

ANNALS OF BOTANY

EDITED BY

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The Evolution of Species in Ceylon, with reference to the Dying Out of Species.

BY

J. C. WILLIS, M.A., Sc.D.

With two Figures in the Text.

IN a paper recently published¹ I have brought forward conclusions which have such far-reaching bearings upon many branches of botany (and probably of zoology also) that it will be well to re-enunciate them in connexion with the further deduction here made from the figures, and which was briefly indicated in that paper, that there is little evidence to show that any species of Angiosperms are dying out.

In a recent paper² upon the Podostemaceae and Tristichaceae I have endeavoured to show that these families, living as they do (and must always have done) under perfectly uniform conditions, cannot owe their evolution to Natural Selection. At the same time they show wide and extraordinary distinctions between species and genera, of the ordinary 'Linnean' type.

In a further paper³ on the origin of these families I have endeavoured to show that they must have arisen from land plants growing at the sides of the streams, and in any case that the first change necessary to give rise to their ancestral forms must have been a 'large' change, which could not therefore have been due to Natural Selection.

In a series of papers published in Ceylon⁴ from 1906 to 1911 I have devoted attention to the very interesting endemic species of that island, and have endeavoured to show that they cannot be regarded as local species owing their origin to the operation of Natural Selection in response to local needs or conditions. These endemic species are not a casual

¹ The Endemic Flora of Ceylon, with reference to Geographical Distribution and Evolution in general. Phil. Trans., B, vol. ccvi, 1915, p. 307.

² On the Lack of Adaptation in the Tristichaceae and Podostemaceae. Proc. Roy. Soc., B, vol. lxxvii, 1914, p. 532.

³ The Origin of the Tristichaceae and Podostemaceae. Ann. of Bot., vol. xxix, 1915, p. 299.

⁴ Six papers in Ann. Perad., vols. iii-v, 1906-11.

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assortment of species occupying spots characterized by special local conditions, but are distributed in the island according to certain fairly definite rules, which obtain equally well in other parts of India and the East, and which I found with much interest to hold also in the case of the numerous endemic species of the state of Rio de Janeiro. These rules, as I have already indicated, and shall endeavour further to prove below, appear to me entirely out of harmony with the current idea that the great differences in the geographical distribution of species are largely due to the operations of Natural Selection. When one works with any number exceeding, say, 15 or 20 allied species of similar distributional origin, one finds that any one group behaves like any other group.

In one of the papers referred to¹ I published the statement that the Ceylon endemic species were rarer than those of wider distribution that occurred amongst them, and made up my mind to enumerate the whole flora in this respect at the first opportunity. This arose with the publication of my 'Revised Catalogue of the Ceylon Flora', and at the same time it struck me that the evidence I was collecting would be rendered much more conclusive were the non-endemic species divided into two groups, those found also in Peninsular India, and those with yet wider dispersal than this.

Marking the species in the Catalogue thus, and entering for each the degree of rarity given by Trimen, who in his great Flora of Ceylon divides all species into six classes—Very Common, Common, Rather Common, Rather Rare, Rare, and Very Rare—I had not done many pages before I realized that I had come upon a general law, which shows as clearly in the figures as does Mendel's Law in any table of results of crossing. Pages 11 to 15 of the Catalogue, for instance, show the following:

TABLE I.

<i>Ceylon spp.</i>						<i>Ceylon-Peninsular-Indian.</i>						<i>Wider.</i>					
						<i>Total.</i>						<i>Total.</i>					
1.	VC	—	—	—	1	1	—	1	—	—	1	2	4	3	2	3	4
2.	C	1	—	1	1	5	—	—	—	—	1	1	5	8	7	3	3
3.	RC	—	1	—	2	5	8	—	1	—	3	4	2	3	6	3	—
4.	RR	—	—	—	—	3	3	—	—	—	—	—	3	2	1	—	—
5.	R	2	—	1	2	3	8	—	1	1	1	1	4	—	2	1	2
6.	VR	8	2	1	2	4	17	—	—	3	—	3	3	3	2	1	3
																	9

If VC (Very Common) be marked 1 and the others up to VR 6, we may easily calculate the average rarity by multiplying the total under each head of VC, &c., by the mark for that head, and dividing the grand total by the total number of species. This shows that the mean rarity (for these

¹ Some Evidence against . . . Natural Selection, *Ann. Perad.*, vol. iv, 1907, p. 12.

five pages) of the Ceylon endemics is 4.5, of the Ceylon-Peninsular-Indian species 3.8, and of the widely distributed species 2.8, in figures running from 1 to 6.

The first dozen pages were amply sufficient to show that a general law was making its appearance, and I went on eagerly to the end, when the grand total showed the figures already published, and which may be quoted again, with the addition of the percentages calculated crosswise.

TABLE II.

<i>Ceylon.</i>		<i>Ceylon-Peninsular-India.</i>		<i>Wider.</i>	
VC	19 spp. 6.66 %	45	15.79 %	221	77.54 %
C	90 13.43	118	17.60	462	68.95
RC	139 25.04	103	18.55	313	56.39
RR	136 31.70	84	19.58	209	48.71
R	192 46.26	64	15.42	159	38.31
VR	233 51.20	78	17.20	144	31.65
	809	492		1508	
Rarity	4.3		3.5		3.0

In other words, the widely distributed species are by far the commonest, and much commoner than the mean of the whole flora, which is obviously 3.5, the mean between 1 and 6. The species found also in Peninsular India, i.e. roughly as far as a line drawn from Bombay to Calcutta, are next most common, with the mean rarity of the whole flora, and the Ceylon endemic forms are very much rarer than this (4.3). This is a very important result, and it will be well therefore to point out how the figures have been arrived at in Trimen's Flora, and that they are quite unassailable.

A great number of species in Ceylon are found only in one small locality, not exceeding a few miles in diameter. These Trimen classifies as Very Rare. A number of localities of such endemic species are shown in the first of the maps given with this paper. So far as my personal experience of such species goes, they are not only confined to a very small locality, but are most often rare within that actual locality. The next stage is Rare, and examination of Trimen's localities (which are all supported by specimens in the herbaria of Peradeniya and Kew) shows that such species occur in roundish areas of from 10-15 to 30 miles or so in diameter, averaging perhaps 24. It is almost needless to remark that cases occur in which it is a matter of individual choice whether a species shall be looked upon as VR or R. Fortunately for this work, therefore, all the decisions (except for Gramineae) were made by Trimen himself. The next higher stage is Rather Rare, which implies a roundish area of about 50 miles in diameter. Not only is the area larger, but the plants in general seem to be commoner in it, and the same may be said with regard to Rather Common, where the area occupied

is yet larger again. In the case of Common, a species is usually found to occupy the whole area suited to it (the island shows Wet and Dry, Warm and Cool zones), and is common therein, whilst Very Common is the same as to area, but the species is yet more abundant. These figures are the result of over seventy years' work by many excellent botanists, and it is not to be expected that more than perhaps one per cent. of them will be found to be erroneous. In any case, as they are based on actual herbarium specimens, it is impossible to *lower* a species in the classification, and consequently the 'wides' must remain much more common than the average of the flora.

In the second place, even if some could be shown to be erroneous, the figures are so numerous that a few alterations would make no difference whatever. Ninety 'wides' might each be lowered a class and yet leave the rarity 3.0. To equalize the endemics and the 'wides' would need 687 alterations in the former, each raising a species one class in the list, and 699 in the latter, each lowering a species one class, which we have just shown to be impossible. The mere fact that the figures come out in such remarkable arithmetical progression along the scales shows that they must on the whole be accurate, for the chances against such an arrangement turning up accidentally are inconceivably great.

Now not only do the grand totals show these figures of rarity for the three groups into which we have divided the Ceylon flora, but (as might be expected from the way in which the law shows, almost page by page of the Catalogue) the figures for each family show the same thing, down to families with 14 endemic species, and the figures for the groups of families containing 12, 11, 10, 8 or 7, 6 or 5, 4 or 3, and 2 or 1 species respectively. These figures are given in detail in Table VI of the Phil. Trans. paper, and it will suffice to quote here the actual mean rarity of the endemic species in all these families or groups, which gives the remarkable figures 4.4, 4.3, 4.9, 4.4, 4.3, 4.5, 4.7, 4.4, 4.0, 4.2, 4.1, 3.9, 4.5, 4.5, 4.1, 4.5, 4.4, 4.2, 3.9, 4.3, 4.0, 4.0, 4.1. If we arrange these in numerical order, we get 3.9, 3.9, 4.0, 4.0, 4.0, 4.1, 4.1, 4.1, 4.2, 4.2, 4.3, 4.3, 4.4, 4.4, 4.4, 4.4, 4.5, 4.5, 4.5, 4.5, 4.7, 4.9.

Such numerical results as these call for immediate explanation, if such be possible. One cannot pass them by as of no importance, as has been the custom with the usual statistics of geographical distribution, which give so many per cent. of Leguminosae, and so many of Orchidaceae, &c., as occurring in the locality under consideration. But to explain them in harmony with the theory of Natural Selection appears to me quite impossible. The further explanation which I put upon them is open to dispute, but the facts themselves are incontrovertible, and, so far as I can see, are very seriously out of accord with Natural Selection. And if the Ceylon flora cannot be explained upon that theory, it at least raises

a grave doubt as to the applicability of the theory in general, for there is not the least reason to suppose the Ceylon flora or the Ceylon conditions to be unique or isolated in any respect.

The interpretation which I put upon the facts is simple. As all the plants, and all the families, behave alike, it is evident that their grouping and distribution must be the result of a cause which acts upon all with practically even pressure. Now Natural Selection could not do this, for in its essentials it is of a differentiating nature. The only cause that I can see which thus acts evenly upon all is *age*, and I am inclined, therefore, to think that the area occupied by any given species at any given time, in any given country, is to a large extent an indication of the age of that species in the country (not its *absolute* age, which has nothing to do with the question, so far as I can at present see). The widely distributed species, which must on the whole be the oldest, are the commonest, the Ceylon-Indian next oldest and next commonest, and the endemics, the youngest, are the rarest. In the case of a group of, say, twenty species of similar distributional origin, and the same family, as we have pointed out above, this is certainly extremely probable, but of course in the case of any single species numerous disturbing influences come into play. And it will be well to point out specifically that these remarks only apply as yet to the angiospermous species, and are based only on the flora of Ceylon, though the close similarity that I have observed at Rio de Janeiro leads me to believe that they apply generally to most floras.

Still more true, in general, is this statement as regards those genera which contain a number of species, than as regards the actual species themselves. In two previous papers¹ I have gone into this question from the point of view of general geographical distribution, and may refer to them here.

In other words, on the view of things thus propounded (which appears to me to have great probabilities in its favour, besides possessing the advantage of explaining numerous as yet unexplained facts in a simple way), endemic species confined to small areas are really *species in the earlier stages of spreading*, and, given time enough, they might ultimately be found covering large areas. Endemic species (all species are on this view endemic when young) begin as VR in some given country, and gradually extend their area, passing upwards through the stages R, RR, RC, &c.

Already these views are meeting with numerous objections, and it will be well to deal with some of these in this paper. The first objection, that the endemic species are on the whole the oldest, and not, as I maintain, the youngest, is easily disposed of by a little consideration. Great numbers of well separated endemic species occur in such genera as *Ramunculus*,

¹ The Geographical Distribution of the Dilleniaceae, as illustrating the treatment of this subject on the Theory of Mutation. Ann. Perad., vol. iv, 1907, p. 69; Phil. Trans., l. c., p. 335.

Polygala, Garcinia, Stellaria, Impatiens, Rhamnus, Vitis, Crotalaria, Desmodium, Poterium, Begonia, Dipsacus, Vernonia, Senecio, Symplocos, Swertia, Ipomoea, Fusticia, Coleus, Scutellaria, Piper, Loranthus, Ficus, Dendrobium, Habenaria, Smilax, Areca, Eriocaulon, Carex, Panicum, &c. It is absurd to suggest that all these genera commenced in Ceylon, and yet this must have been the case if the endemic species are the oldest, unless one imagine that these genera commenced polyphyletically at the numerous places where they exhibit endemic species.

The chief objection to my views comes from the supporters of Natural Selection, and is simply a restatement of their position (which as yet lacks proof) that endemic species are local species developed in response to local needs or conditions. I have already dealt with this question in a series of previous papers, but it will be well to add further arguments here, and especially an arithmetical argument which appears to me of a very conclusive nature.

We have seen above that all the families with fourteen or more endemic species, and all the groups of families with fewer, agree very closely in the degree of rarity of those species, which only varies between 3.9 and 4.9. The mean rarity of the endemic species, from which no family departs very far, is 4.3. Now examination of the areas occupied by the various classes in Trimen's Flora shows that Rather Rare species occupy on an average an area of about fifty miles in diameter, so that a rarity of 4.3 would indicate that the average area occupied by an endemic was about forty miles in diameter. Now in such an area, especially in the hilly south-west of the island, where the bulk of the endemics occur, it is impossible to talk of local conditions, for it includes every kind of soil, great range in local composition of flora, great differences of climate, and many other variations. This simple consideration alone makes a very strong case against Natural Selection. As I have elsewhere pointed out, why should a pinnate leaf suit one valley, and a simple one the next valley to it, in the same genus?

As Table II shows, the endemics increase in number down the scale from 19 Very Common to 233 Very Rare, while the species of wide distribution go in the opposite direction, and those of Ceylon and Peninsular India are fairly evenly distributed. Not only so, but in general, as we have shown, all the families with fourteen species or more, and all the groups of families with fewer, show the same thing.

We may analyse the figures of Table VI of the Phil. Trans. paper, and exhibit the distribution of the endemics within each family in point of rarity, when we obtain the very striking result here shown :

with reference to the Dying Out of Species.

TABLE III.

Family.	Endemics.	VC	C	RC	RR	R	VR	Rarity.
Orchidaceae	78	—	3	19	18	20	18	4.4
Rubiaceae	71	6	6	11	8	17	23	4.3
Dipterocarpaceae	47	—	3	2	8	15	19	4.9
Euphorbiaceae	45	—	3	8	12	9	13	4.4
Acanthaceae	39	—	2	11	6	11	9	4.3
Melastomaceae	36	—	3	6	5	11	11	4.5
Gramineae	31	1	1	3	6	10	10	4.7
Myrtaceae	30	1	4	5	4	3	13	4.4
Lauraceae	23	—	5	5	1	7	5	4.0
Anonaceae	21	—	1	7	3	6	4	4.2
Compositae	19	1	4	2	3	3	6	4.1
Geraniaceae	18	1	2	5	3	3	4	3.9
Scitamineae	17	—	1	5	—	6	5	4.5
Styraceae	17	—	—	4	4	4	5	4.5
Anacardiaceae	15	—	5	1	1	3	5	4.1
Araceae	14	1	1	2	1	4	5	4.5
Total	521	11	44	96	83	132	155	4.4

All the families have some endemic species under every head from C to VR, with only two exceptions in eighty cases (RR in Scitamineae, and C in Styraceae). And the numbers on the whole increase in each family from top to bottom of the scale.

Thus, with five-eighths of the whole number (809) of endemics, we find a wonderful likeness among the different families in the proportions of endemics in the classes C, RC, &c. The families with many endemics show greater average rarity than those with few, as has already been pointed out (cf. Table XVI of previous paper). It is not possible for the Natural Selectionist even to derive consolation from the remaining families with small numbers which are lumped together in Table VI just quoted. If we place them according to rarity, we get a simple table of variation of the usual trial and error pattern:

TABLE IV.

Rarity.	Families.	Species.	No. of spp. per family. ¹	Marks.
1.0	1	1	1.0	1
1.0-2.0	7	9	1.2	17
2.1-3.0	6	19	3.1	53
3.1-4.0	20	74	3.7	270
4.1-5.0	32	161	5.0	736
5.1-6.0	9	24	2.6	132
	75	288		1209

Or if we analyse them according to the classification of their contained endemics, we get,

TABLE V.

Families.	Endemics.	VC	C	RC	RR	R	VR	Rarity.
3 with 12 spp.	36	—	6	4	6	5	15	4.4
4	11	44	1	5	7	9	11	4.2
2	10	20	2	5	—	5	2	3.9
7	8 or 7	52	1	7	9	7	12	4.3
6	6 or 5	32	1	4	6	8	9	4.0
15	4 or 3	47	2	9	8	7	8	4.0
38	2 or 1	57	1	10	9	11	13	4.1
Total	288	8	46	43	53	60	78	4.1

¹ This shows very clearly that the wider deviations are mainly in the smaller families.

—a table exactly similar to Table III above, but with still lower average rarity (cf. Table XVI, Phil. Trans. paper).

The same fact shows if one analyse the tables of rarity for the species of those genera which contain ten or more endemics, this gives,

TABLE VI.

	VC	C	RC	RR	R	VR	Total.	Rarity.
Doona ¹	—	1	1	2	4	3	11	4·6
Stemonoporus ¹	—	—	—	2	4	9	15	5·4
Impatiens	1	1	3	3	3	4	15	4·2
Semecarpus	—	4	1	—	3	5	13	4·3
Eugenia	1	4	5	4	2	13	29	4·4
Memecylon	—	1	4	4	5	7	21	4·6
Hedyotis	2	1	—	—	5	8	16	4·8
Symplocos	—	—	4	4	4	5	17	4·5
Strobilanthes	—	1	7	5	4	8	25	4·4
Amomum	—	—	3	—	5	3	11	4·7
	4	13	28	24	39	65	173	4·5

The rarity varies only between 4·2 and 5·4, i.e. is never so low as the mean of the whole flora (3·5), nor so high as strictly local (VR = 6). And taking the classes C to VR, there are only eight cases out of fifty where there are no representatives of a genus (3 C, 2 RC, 3 RR).

We may even take the genera with from five to nine endemic species, and get,

TABLE VII.

	VC	C	RC	RR	R	VR	Rarity.
Acrotrema	—	1	—	1	2	3	4·8
Goniiothalamus	—	—	—	1	2	3	5·3
Calophyllum	—	2	—	1	2	2	4·2
Dipterocarpus	—	1	—	—	3	1	4·6
Shorea	—	—	1	2	—	2	4·6
Vitis	—	—	1	2	2	—	4·2
Sonerila	—	1	—	—	3	4	5·1
Psychotria	—	—	1	—	5	3	5·1
Lasianthus	—	3	1	2	1	2	3·7
Vernonia	1	2	2	2	1	1	3·3
Palaquium	—	—	1	2	2	2	4·7
Diospyros	—	1	—	2	2	3	4·7
Gymnostachyum	—	—	1	1	2	1	4·6
Actinodaphne	—	2	1	—	4	—	3·8
Litsea	—	2	3	—	2	2	3·8
Loranthus	—	1	—	1	2	4	5·0
Phyllanthus	—	—	—	2	4	3	5·1
Glochidion	—	1	2	3	1	1	3·8
Dicotyledons	1	17	14	22	40	37	4·4
Oberonia	—	—	—	3	3	1	4·7
Liparis	—	—	—	1	1	3	5·4
Bulbophyllum	—	—	—	1	—	4	5·6
Cirrhopetalum	—	—	2	2	—	1	4·0
Eria	—	1	2	—	1	1	3·8
Saccolabium	—	2	1	1	—	1	3·4
Habenaria	—	—	1	2	4	1	4·6
Calamus	—	—	2	2	3	—	4·1
Eriocaulon	—	—	1	—	1	5	5·4
Carex	—	2	—	1	—	2	4·0
Gamotia	—	—	1	—	4	1	4·8
Monocotyledons	—	5	10	13	17	20	4·5
Grand Total	1	22	24	35	57	57	4·5

¹ Endemic genera.

Only two genera in this list (*Vernonia* 3.3 and *Saccolabium* 3.4) are equal to or above the average in point of rarity.

One may even go lower than this, and take the genera with fewer than five species. Naturally any one genus may show any figures from VC to VR, but if one add them together in groups one gets the same result as before. We may take all the genera beginning with one letter of the alphabet, and get,

TABLE VIII.

	VC	C	RC	RR	R	VR	Rarity.
Beginning with A	3	9	11	11	12	11	3.9
B	—	4	5	2	10	4	4.2
C	—	5	10	11	13	23	4.6
D	—	1	4	5	3	9	4.6
	<hr/> 3	<hr/> 19	<hr/> 30	<hr/> 29	<hr/> 38	<hr/> 47	

There is no need to follow the comparison through the whole alphabet. It is sufficiently obvious from all these tables that the distribution of the species of the various endemic families and genera obeys a simple law which determines that the numbers under the different heads from VC to VR shall be distributed in proportions increasing from the former to the latter. The numbers simply vary about the suppositional numbers in the ordinary way of trial and error. The maximum is always or nearly always at VR.

If one analyse the same tables as to the figures for the Ceylon-Indian and widely distributed species, one finds exactly similar results, but with the maxima differently placed. For instance, the first five orders (i.e. in number of endemics) show,

TABLE IX.

	Ceylon-P.-India.					Wide.				
Orchidaceae	1	6	9	10	5	8	1	6	12	8
Rubiaceae	6	5	3	6	4	1	7	13	9	5
Dipterocarpaceae	—	—	1	—	—	—	—	—	—	—
Euphorbiaceae	4	8	4	3	3	4	4	26	8	7
Acanthaceae	1	7	7	4	7	4	7	9	5	2
Total	12	26	24	23	19	17 (121)	19	54	34	22

For convenience it will be well to arrange these together with the endemics, thus :

TABLE X.

	VC	C	RC	RR	R	VR	Total.
Endemics	6	14	49	44	57	63	233
Ceylon-P.-I.	12	26	24	23	19	17	121
Wide	19	54	34	22	28	13	170

Whilst the maximum of the endemics lies at VR, that of the Ceylon-Peninsular-Indian species lies at C, with no very great tailing off towards VR, and that of the Wides lies very markedly at C, with an immediate and sudden drop to RC and RR.

The mere fact that the numbers representing rarities come out with such simple arithmetical relations seems enough to show that whatever cause is operative in causing such relations it cannot be Natural Selection. So simple an explanation of evolution is impossible, though a more 'mechanical' explanation of its more obvious features than is Natural Selection is called for.

[All the tables of rarity given in my previous paper show the same thing, varying in almost purely 'mechanical' ways. Table VI shows that the rarity of the endemics is much the same for all, taken family by family. Table IX shows much the same thing for genera, and if analysed gives the following figures:

TABLE XI.¹

Rarity	1-2		2-3		3-4		4-5		5-6	
	Genera.	Spp. per genus.	Genera.	Spp. per genus.	Genera.	Spp. per genus.	Genera.	Spp. per genus.	Genera.	Spp. per genus.
Endemic	5	1.2	6	2.0	18	3.7	26	6.6	19	2.6
Ceyl.-P.-I.	18	1.5	16	3.0	24	2.1	13	2.8	3	1.3
Wide	19	1.4	34	6.0	14	6.0	5	2.0	2	1.0

The regularity of the numbers according to the ordinary rules of trial and error is too great to be explicable on any hypothesis of other than a mechanical cause. The larger genera come nearer to the means.

Table XV shows equal mechanical regularity coming out in the distribution of species according to the zones they live in. Tables XVII, XVIII show that endemic genera obey the same rules as endemic species. Table XIX is even more striking, as showing the same rule coming out within each of the single endemic genera *Doona* and *Stemonoporus*.]

When we come to look at these tables, it is evident that the distribution of the endemic species of Ceylon obeys a simple law which determines that there shall be, family by family, and genus by genus, some species under all the heads of classification of rarity from VR up to VC, but that the number shall be a decreasing one, there being ten times as many VR as VC. In every family or larger genus there are usually species under VR, R, and RR, but a few get no higher than this, more stop at RC, yet more at C, and very few reach to VC at all. The law is quite clear from the figures, though of course, like Mendel's Law, it is followed with the usual variations due to the operations of the laws of trial and error.

At the same time, the distribution in Ceylon of the species which are also common to Peninsular India, and that of the species of yet wider distribution, obey exactly similar laws, but in each of these cases the maximum is not at VR, but at C, in the first case with only slight falling off towards VR, in the second case with a very marked decrease.

¹ Maxima underlined.

Now whatever cause makes all these species of this graduated degree of rarity must be a cause which acts equally upon all. Family by family, and genus by genus, all alike obey the same law, and have some species VR, or confined to a very minute area; some R, or confined to an area of about twelve to twenty-four miles in diameter, and rare in that; some RR, or occupying an area of forty to fifty miles in diameter; some RC, with yet larger territory, and commoner in that, and so on. The Ceylon-Peninsular-Indian species, and the widely distributed forms, show exactly the same phenomena, but, as would be expected upon my hypothesis, show greater trial and error variation among themselves. The order of appearance of new species in Ceylon is more likely to be regular than the order of appearance in the island of species from abroad, and new species, confined to small areas, are perhaps more likely to show adherence to the law in detail than more widely distributed species, which may enter the island, for anything we can tell, at several points more or less simultaneously.

It is inconceivable that Natural Selection, which is an agent of essentially differentiating nature, should thus act with uniform pressure upon every family in the flora, and upon every larger, one might say every, genus. The only cause that I can conceive that thus acts is *age*. Young endemic species, and newly arrived species from abroad, show rarity VR, but as time goes on they will creep slowly up to R, and later to even higher stages in degree of commonness. Working upon averages of, say, twenty they all behave alike, but in any individual case of course the rate of progress will be determined by degree of local adaptation, and still more by chance, so that of two species starting on the same day at the same degree of rarity VR, after a certain lapse of time the one may have reached RC, the other only R.

To me this arithmetical argument appears to clinch the matter,¹ but as it may not appeal to those who have not an arithmetical turn of mind, it will be well to put what is much the same argument into a biological dress. Taking from Trimen's *Flora of Ceylon* the first few endemic species of the degree of rarity VR, R, and RR, and marking their position (if VR) or drawing a ring round their recorded localities (if R or RR), we get the results shown in the three little maps (Fig. 1). The localities are not shown with absolute accuracy in these little maps, but nearly enough for the purpose. I have much pleasure in acknowledging the help I have received in identifying the localities from Mr. Frederick Lewis, late of the Land Settlement Department in Ceylon.

Comparing these three maps, the first thing to be noticed is that each is like the others, but with different sizes of area. The VR species cover the map (if the whole 233 were taken) with a pattern of small dots or lines.

¹ So far as Natural Selection is concerned.

In five cases (in this selection) the species has been observed in two localities a long way apart, which are joined by a wavy line. In all probability, as these lines go through the least thoroughly explored parts, the species occurs in localities between, and should really be regarded as R or even RR.

The Rare species cover the map with a pattern of small interlacing circles, which overlap one another in the most complex way, but never coincide, and resemble the rings in a shirt of chain-mail. The RR species

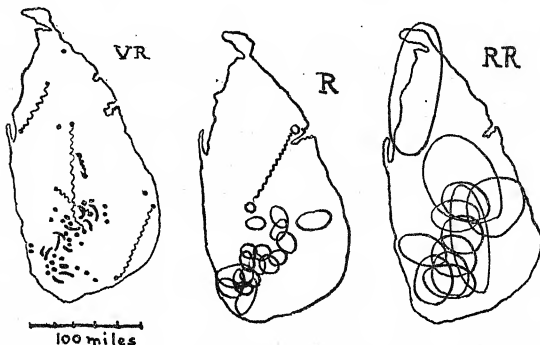


FIG. 1. Distribution in Ceylon of the earlier VR, R, and RR species from Trimen's Flora.

show the same thing but with yet larger circles. The three diagrams may very well be looked upon as exhibiting three stages in the process of gradual spread of species, which commence as VR and go through the other stages in turn.

The second point that shows at once in these diagrams is that the enormous majority of the endemic species are in the wet zone (which comprises the south-western quarter of the island), as has already been pointed out (cf. Table XV of the Phil. Trans. paper).

Now the important point about these distributions, from the Natural Selection point of view, is that nowhere do the areas occupied by the endemic species coincide, except in a few instances among the VR forms, where it often happens that two or three or more occur on the same mountain top. On Nillowekanda, for example, there are found, and there only, *Acrotrema lyratum*, *Stemonoporus reticulatus*, and *Ochna rufescens*; on Ritigala three species; on Hinidunkanda three; on Adam's Peak ten, one

of which extends into the valley of Maskeliya 2,000 feet¹ below. There seems to be something about a mountain top which causes it to be an especially favourable place for the commencement of new species. Nearly every isolated mountain in Ceylon has its own species. On the other hand, the conditions are the same to all intents on these summits, except if averages be taken over many years. And in the large forests, where the conditions must be equally uniform, one does not find the VR endemics coinciding in localities; each has its own.

The R and the RR species show distribution areas forming a kind of chain-mail pattern over the country. No two have the same area of distribution. If we were to find a number of species jointly occupying a certain area, and a number of others occupying a second, it might be possible to say that they were adapted to some special local conditions there existing. But how can such an explanation be brought forward for a chain-mail pattern? What conditions can one find to distinguish four or five areas which overlap one another? At the parts where they overlap, all species must be growing in the same conditions. What then prevents *A* from spreading into the area occupied by *B*?¹ The mere demonstration of the actual distribution of the endemics of Ceylon is almost sufficient to show that no explanation involving response to local conditions can possibly serve to explain it, but that there must be at work some cause which is much more purely 'mechanical'.

In the second place, the number and proportion of endemics are far greater in the wet south-western zone, i.e. in the broken hilly country of Ceylon, than in the flat and uniform dry country which surrounds it to north, east, and south-east. If all the endemics were VR, this would fit in admirably with Natural Selection, for each species could be looked upon as suited specially to its own mountain top or other locality. But unfortunately for this hypothesis, while there are 233 of these VR species, there are also 192 R, 136 RR, 139 RC, and so on in diminishing numbers upwards. Even the R species cover so much ground that they come into a great variety of conditions, and this becomes more marked with each step upwards in the scale. Many RC species, for instance, cover the bulk of the upper montane zone; this is composed of hills, valleys, and plains, of regions of richer and of poorer flora, of granitic and gneissose soils, of higher and of lower rainfall, temperature, &c. How can a species become adapted to such a region in which no two places show the same conditions?

Even if one takes the most local VR species, one does not in reality encounter uniformity of conditions. In one year the climate is wet, in the next dry, and if a form settled, or arose by mutation, in a wet season, how

¹ Of course on my hypothesis there is nothing. Given time enough, *A* may spread into the whole area now occupied by *B*.

did it survive the dry one which followed, unless it was really suited to a comparatively large range. In one year the plant may have to compete only with two or three others, in the next with five or six, and with new arrivals of animals or diseases, &c. If a species such as one of the R or RR forms be suited to local conditions, it must be to averages, and how did a species evolve to suit an average, and when did it decide that the average was fulfilled? Accepting mutation, as one must accept it, it is evident that the parent must have been able to stand the conditions in some variety, and why should it give rise to a local form, which one cannot conceive as any better suited to them (on an average)?

Not only do the endemics as a whole show these increasing areas of distribution on a regular numerical plan, but the single families and the single genera show the same thing, as has already been explained in the preceding argument, and as may be illustrated by the case of *Doona*, a rough outline of whose distribution is given in the accompanying cut. *D. zeylanica* has an area of distribution overlapping all the other species, which show smaller and smaller areas within it. If one set out to explain the distribution of endemic or other species in Ceylon by Natural Selection, one has to explain why every family and genus has approximately the same proportion of areas VR, R, RR, RC, C, and VC, when one can only pretend to talk of local conditions in the case of VR.



FIG. 2. Distribution diagram for the genus *Doona*.

The idea that endemic species were evolved to suit local conditions is based largely upon Wallace.¹ So long as we imagined *islands*—taken as a whole—as their habitats, it was possible to say that the local flora, the local fauna, local soil, local conditions generally, varied for each island, as for instance in the Galapagos. And this idea was encouraged by the fact that the studies of endemism have mostly been made in zoology, and an animal confined to one island will often be able to roam over the whole island. It received its first real and severe shock from the investigations of Gulick² on the local endemic forms of the Mollusca in Hawaii.

But when one comes to try to apply this idea to the endemic plants of Ceylon, which are often very local *within* the island (25,000 sq. miles), one rapidly gets into great difficulties. The climates of two mountain tops in the island only differ in averages, and how do plants evolve to suit an

¹ Island Life, and other papers and books.

² Evolution, Racial and Habitudinal, Washington, 1905.

average climate? The composition of the flora is never absolutely the same round any two plants, unless they happen to be strangers growing in pure forest. No two samples of soil are absolutely the same. And so on. When one comes to the final analysis of the situation and conditions of any given species, one finds that no two individuals are growing under exactly the same conditions, and yet, for the theory of local adaptation to have a foothold, it is necessary that there should be only the very slightest differences between them, though in actual fact these differences are often greater between two plants of the same local endemic species than between two species widely separated in distance and affinity. It is idle playing with words to say, as do many of the supporters of Natural Selection, that there is something there that we do not understand, and that there must be differences in conditions that we cannot appreciate, and differences in adaptation that we do not and cannot perceive. Why should one endemic of Adam's Peak be able also to survive in the widely different conditions of the Maskeliya valley, 2,000 feet below, and another be confined to the Peak itself, whilst a third ranges also to a second mountain peak? Why should one range some distance down the Peak into different floras and climates, while another is confined to the summit? Why should one species in the Singhe Raja forest, where on an average the conditions must be uniform, occupy an area of 100 metres in diameter, another one of 1,000, a third one of 10,000? No other explanation than that which I have brought forward can answer questions like this, which may be put by the dozen, without invoking incomprehensibility, whereas that which I have given, that the dispersal of a species depends chiefly on its age, and that the endemic forms are simply young species not yet widely dispersed, recommends itself at once by its simplicity and wide applicability, and by the fact that as yet nothing can be brought up against it which is incapable of explanation, though doubtless, as with nearly all theories, it will prove insufficient later in its history.

No evidence has ever been brought forward to prove that local species are adapted to local conditions; it is simply an hypothesis, upon which it is impossible to explain such cases as the seven species of *Castelnavia*, living in successive cataracts in the same river, without any other forms to compete with, and with all the conditions of substratum, circumambient medium, climate, &c., absolutely the same for all, both now and in all previous time.

The families Tristichaceae and Podostemaceae, as I have shown in another paper,¹ supply a crushing rejoinder to those who maintain that specific differences are due to local differences in conditions. They grow in such situations that there cannot be, nor ever have been, any local differences between them, and yet they show greater differences in morpho-

¹ On the Lack of Adaptation in the T. and P. Proc. Roy. Soc., B, vol. lxxvii, 1914, p. 532.

logical structure than any other families. What is there in the differences between life on rocks in India and life on rocks in Brazil, in both cases without external competition, and submerged in running water, to cause the Indian species to develop flattened roots which adhere closely to the substratum, while the Brazilian species have flattened secondary shoots which do not adhere to it? The advocates of adaptation say that the water strain must be greater in India. This explanation was good until I had personally investigated some 120 waterfalls and rapids in which these plants grew in both countries, but there can be no doubt that the Brazilian forms, with far less holdfast, often grow in far more rapid water. Since writing that the fastest water in which I had found any was in the Rio Piabanha, four miles an hour, I have found a large leafy *Mourera*, a *Tristicha* (probably *hypnoides*), and others growing in the cataract of Ronca Pao, near Cantagallo (state of Rio), where the water was flowing at least eight miles an hour. They were growing at the bottom of a great water-slide of perhaps 150 feet in height, with a slope of 30°. Where the water struck the ledge on which they were growing, it spouted up above the general surface. Never before have I seen plants in such an extraordinary situation.

No explanation of such facts as we have been bringing forward in this paper can be produced by the advocates of Natural Selection, who are content to say that there must be differences in adaptation imperceptible to us, and which we do not and cannot perceive. After forty years no one has even been able to make a reasonable suggestion as to what these differences may be. But if it be admitted that dispersal goes with age, the whole is at once clear, and the way is open for innumerable new investigations.

Another objection to my views admits the greater rarity of the endemic species,¹ and states that the mere fact of wide distribution makes a species commoner even among the endemics of a given country, which are (on the old theory) developed to suit that country. This position has always appeared to me an unsound one, and must have arisen from the necessity of explaining the fact that this is often the case, and from thinking chiefly of animals, which are able to range about over considerable areas. So far as the widely distributed species are concerned, Ceylon must have been stocked with them from the plants which were growing at or close to the point of junction with India. But why should the mere fact that a widely distributed species is growing near Tuticorin enable its offspring to spread in Ceylon more widely than a species evolved in the island? Why should the mere fact that the ancestors of *A* (a widely distributed species) have lived at all the various stages on the road from, say, Delhi to Tuticorin, make *A* more common in the island than *B* (a Ceylon-

¹ But does not explain why they are (family by family and genus by genus) graduated in rarity from VC down to VR,

Peninsular-Indian species), whose ancestors only came from, say, Hyderabad or Poona? If the plants at Tuticorin were in the habit of ranging over the whole area occupied by the species this might be conceivable; but as things actually are, this hypothesis, which at present holds the field (though sadly injured), involves the bold assumption that the plant living at Tuticorin carries in itself the accumulated experience of many different climates and conditions that have been passed through by its ancestors. And for this we have absolutely no warranty whatever, whilst, as pointed out elsewhere, the actual conditions, even for the endemics with the most limited area of distribution—an acre or two—are never the same for two plants of the same kind, nor for the same plant in two consecutive seasons.

If we do not make this assumption, we have to make the almost equally bold one that the greater distributional areas of some species depend upon the fact that they were born with greater 'adaptability'. And here we meet at once with the difficulty of explaining why the species endemic to South India as well as Ceylon—often only to a small part of South India, e. g. the Nilgiris—should be more adapted to Ceylon than those endemic to it only. As Table II shows, 45 out of 492 such species have become very common, against only 19 out of 809 of the Ceylon species proper. And of the 'wides' no less than 221 out of 1508 have become very common. Such facts would go to show that there was nothing to be gained by having local endemic species, which could not become so common as those which came from a distance, and would also indicate that adaptability was in some way bound up with origin upon a large area. The wides are the most, the Ceylon-Indias the next, and the Ceylons the least adaptable to the *Ceylon* conditions.

If it be assumed that the 'wides' were originally developed over the whole of large areas, we have to explain why such a fact should make the descendants of any one of a given species more adaptable to Ceylon, especially when we remember that in the case of widely distributed species we usually find that there are many local varieties. Does each local variety carry in itself the adaptability to spread into new areas with great success?

The advocates of the old view are faced by a very difficult question when the matter is reduced to figures as in my previous paper. Why should a species that ranges over Ceylon and Peninsular India be commoner in Ceylon than one that only ranges over Ceylon? Because it has a wider range, they reply. And why should one that ranges over a larger area be commoner yet? Because it has a still wider range, is the only reply they can make. This is simply an appeal to ignorance. They cannot suggest any convincing reason why mere wide range should involve greater commonness, unless it be simply greater age within the country, as I maintain. If mere wide range involve greater commonness, why are so

many wides VR and some endemics VC? If we put it all down to age we get a simple and perfectly reasonable explanation, without making any appeal to ignorance.

All these facts, taken together, go to show that whether the endemics be regarded as increasing in number, or dying out, their growth (or decay) is almost purely mechanical, and cannot therefore be explained on any other than a mechanical hypothesis. For this hypothesis I have adopted that of age. It is, however, absurd to imagine that the endemic species are older than the others, and consequently, if they are dying out, we have to explain the remarkable fact that the youngest species are dying out, and that mechanically, as rapidly in one family or genus as another.

The whole goes to show that evolution, like so many other things, eludes our grasp just when we think that we have at last found a method which will give us a close insight into it.

But if my interpretation be accepted—and it is very hard to see how any other can be found—great changes must follow, and in the present and succeeding papers I shall indicate a few of these. It may be noted here that we practically get rid of the idea of variation in a species until it has attained considerable age; there is no room for it in the small number of individuals with which the species begins.

Evolution is thrown back a stage, and we come once more, after many wanderings, back to the definition of Linnaeus, 'species tot numeramus quot diversae formae in principio sunt creatae', but substituting *evolutae* for *creatae*. The species, in fact, in the Linnean sense, comes first, and varies into the innumerable varieties which may ultimately exist, later.

One may further test the validity of using the figures for rarity as they were used in the preceding paper, by checking various other problems where one can with reasonable probability predict the answer. Thus, for example, it is obvious that on the whole parasites and Saprophytes can only come later than the other plants of the Angiosperm flora. With the figures for the Ceylon flora, we find that the widely distributed species of these groups show a rarity of 3·8, against a mean of 3·0, indicating their greater youth.

Climbers, again, must on the whole be younger than the rest of the flora. Testing this, we find 179 species of widely distributed climbers, with 583 marks, numbers large enough to give a fairly accurate result, which shows rarity of 3·2, or again younger than the rest.

In the case of water plants there is no reason to imagine them necessarily any later in arriving than the land plants, and in fact they show,

TABLE XII.

Dicotyledons of wide distribution	34 with 87 marks	2·5
Monocotyledons	26 98	3·7

These figures go to show that the Dicotyledonous water plants are very old in Ceylon, as indeed one would expect, while the Monocotyledonous species are much more recent, even than the average of the whole flora.

We have now to go on with the calculations from these figures, to determine whether any other information of value may be extracted from them, and first we shall deal with the question whether they show any indication that any of the angiospermous species of the Ceylon flora are dying out. The theory that about as many species are dying out as are in course of extension is still more or less implicitly held as an article of faith.

We may refer to Table II, at the beginning of this paper, which gives the actual numbers of species under each of the different heads of rarity, with the percentages.

As there exist in Ceylon 455 VR species, which are all confined to very small areas, often one single mountain top, it is evident that for the present the best classification of the flora is into six approximately equal groups, each containing about one-sixth of the total, or 468 species. But whether this grouping would always remain the same for all future time is another question, to which the observed facts give a clearly negative answer. The oldest group of species in the flora—those of wide distribution—show numbers increasing upwards in the scale; the next oldest show numbers fairly evenly divided along it, and the youngest group, the Ceylon endemics, show numbers increasing downwards. But it must be remembered that the species of wide and of Ceylon-Indian distribution were cut off at a certain period, subsequent to which no more could enter the island. On the other hand, so far as we know, there is nothing to prevent new endemics forming to-morrow, and the number of endemics would in all likelihood increase with the increasing number of the other species; hence the table with numbers increasing downwards. The view which I take of the history of the first two groups is that the numbers that first entered Ceylon would be small, and would increase as time went on and the number of species on the Indian peninsula increased. Then as Ceylon began to be cut off, the communicating land would narrow, and the number of species arriving across it would gradually decrease. This may be very roughly indeed represented by the following table (XIII). Having no knowledge of how rapidly the proportion of species crossing to Ceylon increased, of how rapidly the number of Ceylon-Indian forms grew, nor of how rapidly the communication was cut off; and remembering that the early arrivals probably went more quickly up the scale than the later, and that there were probably such differences in adaptation, and in the chances that befell, that some would never rise above some definite level in the scale short of VC, it is evident that much greater mathematical skill than I possess is needed to construct any sort of diagram approaching accuracy, if indeed such were at all possible in the present very limited state of our knowledge as to the past history of Ceylon and its vegetation.

TABLE XIII.

VC					1	3	6	10	15	21	
C					1	2	3	4	5	5	
RC				1	2	3	4	5	5	4	
RR			1	2	3	4	5	6	5	4	
R				2	3	4	5	6	5	3	
VR	1	2	3	4	5	6	5	4	3	2	
Indian connexion cut											
	A	B	C	D	E	F	G	H	I	K	L

Column A represents the very earliest stage of all; the first widely distributed species have arrived, and are still Very Rare. In column B these have become Rare, and more numerous species of wide distribution are arriving. And so it goes till in column F the first arrivals have become Very Common. The connexion with India is now supposed to be severed, but the Very Rare species will not as a matter of fact rise to Rare for a long time. In column G we get a rough representation of the state of the Ceylon-Peninsular-Indian species at the present time, whilst column F represents the endemics, and column H the species of wide distribution.

Supposing that the supply of endemics could also be stopped, so that the column of rarities representing them would gradually take the form of that representing the Ceylon-Indian species, and later that of the widely distributed species, one might ultimately find that the grouping into six equal classes at present employed would have to change gradually to one in which the greatest (and an increasing) number were VC, and the least (and a decreasing) number were VR. The class VR, as at present understood, would gradually disappear, or be enormously reduced. Or, if we continued to divide all the species into six equal classes, then VR would gradually come to include what R now stands for.

As time went on, it is conceivable, if not probable, that VC as a class would also disappear, for the numbers actually existing of the species now in VC would be reduced as other species entered the class (for one can only get a certain number of plants upon a certain space of ground). Later the classes C and R might also disappear, and the whole flora become either RC or RR.

But leaving all this out of consideration for the present, it would appear that the evidence distinctly points to the supposition that as time goes on species rise in the scale of classification (VC to VR in equal classes) at present adopted, and that there is no evidence that any are dying out.

On any theory of dying out of species, it is obvious that on the whole it must be the oldest that are disappearing, but the figures lend no support to such a hypothesis. They would rather go to show that if any are dying out, it is the endemics—a very remarkable result whether one consider them as the youngest species or as the species specially developed to suit the local conditions of Ceylon. In what way the figures we have given are to be reconciled with any theory of dying out of species I fail to understand.

But if no species of the Ceylon flora, which is without doubt a very old one, are dying out, it is unlikely that any are dying out elsewhere.

One may arrive at the same conclusion by studying the actual composition of the floras of different regions of the world, as has fallen to my lot to an unusual degree. Ceylon, though equatorial in position, has but a small flora (2,809 species) compared with the islands of the eastern peninsula of India, Java having, so Dr. Stapf kindly informs me, 5,067 recorded to date. This has always been a difficult matter to explain, and the Natural Selectionists have had two rival hypotheses, which it may be pointed out are mutually contradictory. The first is that Ceylon has a less 'tropical' climate than Malaya, having greater extremes of wet and dryness and of heat and cold. The second is that Ceylon has but a poor soil, with no variety in it, it being all the product of decay of gneiss and granite. On the first of these hypotheses the less variety in species is put down to greater variety in conditions, on the second to less.

The first objection which occurs to one is that South India, with the same geology and a more variable climate, appears to have more species than Ceylon, and this is most remarkably supported by the case of the state of Rio de Janeiro in southern Brazil, which has just about the same area as Ceylon, has the same geology and soil, and, except in the narrow coastal belt, has a greater variety of climates, at any rate as regards heat and cold. The flora of Rio is something enormous, and as much richer than that of Java as the latter is richer than that of Ceylon. Dr. Löfgren, my late colleague, than whom no one better knows the flora of Brazil, estimates the flora of the state of Rio at 7,000-8,000 species. He calculates the flora of the single mountain of Itatiaya (10,000 feet), most of which is within this state, at 7,500 species.

I explain this variety in the size of the floras mainly by the fact that the state of Rio has always, so far as geological evidence goes, been attached to large continental areas and is itself of enormous age. It has thus been open to the invasion of great numbers of foreign species, whilst its own mountainous configuration has tended to the development of large numbers of endemics within the state, just as in Ceylon every hill (and here also every little island off the coast) seems to have its own forms. Only a few days before I left Rio, Dr. Löfgren found on one of the nearer islands a most remarkable new species of *Rhipsalis*, which one could only describe as a pendulous shrub or tree, its stem being about four inches in diameter.

From these and other similar facts I draw the conclusion that the number of species in a country depends upon its age from the time of its last submergence, and upon whether it has been attached to large areas with many species during most of its history, or whether it has been cut off at an earlier or later date. Species, or at least the majority of them, do

not appear to die out except by accident. A very small accident may kill out a species while at or below the stage represented in the Ceylon classification by VR, whilst it will need a geological submergence or some such accident to kill out one represented by VC.

Whether under exactly equal conditions of age, attachment to other areas, and favourable climates, &c., a tropical area would have more species than one in the temperate zone of equal size must remain an unsettled question. Most of the evidence on which we have relied for an affirmative answer must now be regarded as incapable of bearing such a load, and the rich floras of South Africa and West Australia contradict the assumption. As yet we know of nothing that can be adduced as a reason why almost limitless species should not survive on an area with reasonably good climatic conditions. There is no evidence whatever that any of the angiospermous species of the Ceylon flora are dying out, and from analogy we may imagine this to be generally true.

SUMMARY.

The paper is a continuation of previous papers in which, among other things, I have sought to show that Natural Selection has but little to do with the geographical distribution of species or the areas they occupy, and that the area occupied at any given time in any given country depends mainly upon the age of a species in that country (not its absolute age).

The figures of rarity of the Ceylon flora, derived from the statistics extracted from Trimen's Flora of Ceylon, are considered, and it is shown in the first place that they are incontrovertible, being much too numerous and too well worked out. The rarity of the endemic species (in figures going from 1, Very Common, to 6, Very Rare) is 4.3 or very close to that figure (Table II), that of the species found also in Peninsular India is 3.5, and that of the species of wider distribution is 3.0.

Not only do the grand totals show these figures, but they come out family by family. Natural Selection cannot produce a result like this, acting with equal pressure on every family, and I therefore attribute the distribution of species (taking them in groups of twenty or more) to age.

Many objections are being raised to these views. The first, that the endemic species are really the oldest and not the youngest, is easily disposed of by the consideration that they belong to the same genera. The second, a restatement of the contention of the supporters of Natural Selection, that they are local species developed to meet local needs or to suit local conditions, is met largely by an arithmetical argument.

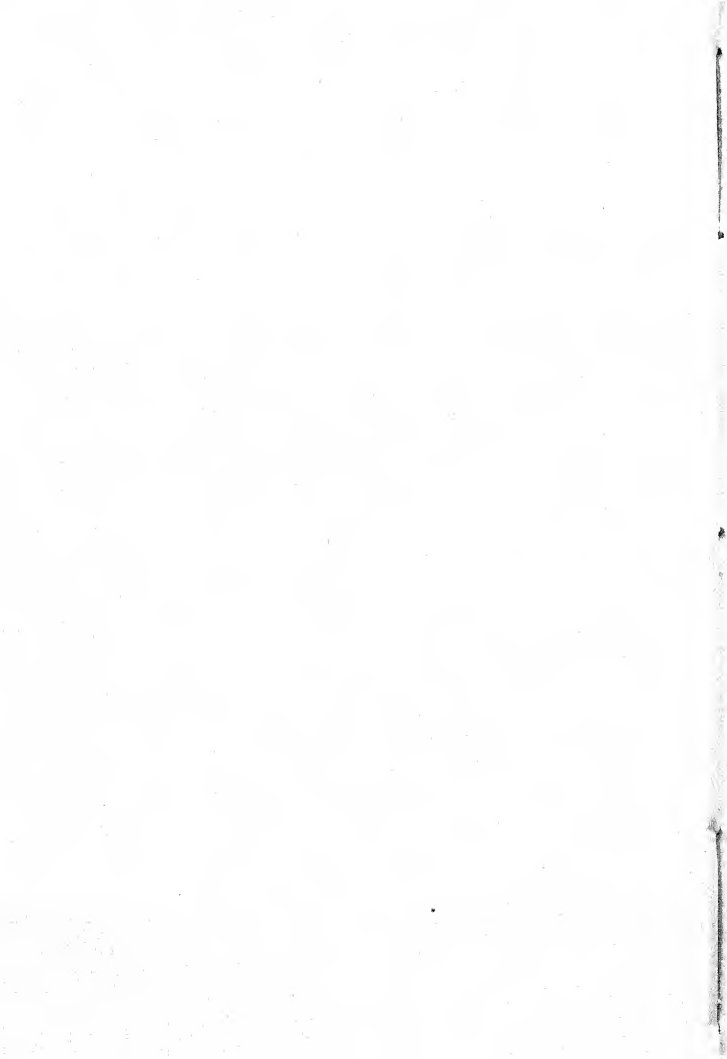
Not only is the rarity much the same in every family, but all families show some species under every head from Common to Very Rare, and in increasing numbers (Table III). This holds for all families with fourteen or more endemic species, and for all groups of families with fewer. The

same facts show in all the genera with more than ten species (Table VI), with from five to nine species (Table VII), and even in the genera with less than five, when taken together in groups (Table VIII). Always the maximum is at or near VR. In the same way the Ceylon-Indian and Wide species show parallel figures, family by family and genus by genus, but with maxima at Common (Table X). It is inconceivable that Natural Selection (a differentiating agent) should thus act with uniform pressure on every family and genus; the only factor that to me seems satisfactory is age.

The same argument is then put into biological dress, with slight alterations, and maps of Ceylon are given showing a number of Very Rare, Rare, and Rather Rare species. The VR areas are small dots scattered over the map, the R's little rings, and the RR's larger ones. But all are scattered, and the circles overlap like the rings in a shirt of chain mail. Now it is impossible to find conditions varying in such a way as to cause such distribution as this, and it is much simpler to look upon the area occupied as an indication of the age.

A general discussion is then given, and it is shown that the advocates of Natural Selection do not satisfactorily explain such facts, but rather pass them over as incapable of explanation. Other objections to my views are also dealt with.

The question of dying out of species is then considered, and it is shown that the figures of distribution of the Ceylon plants give no reason to suppose that any angiospermous species are dying out at the present time, a supposition which is borne out by a comparison of the floras of Ceylon, Java, and Rio de Janeiro.



Some Experiments on the Influence of Temperature on the Rate of Growth in *Pisum sativum*.

BY

I. LEITCH, B.Sc.

With Plate I and ten Figures in the Text.

THE first work of importance on the subject of the relation of temperature to growth-processes in higher plants is that of Sachs ('60), on the effect of temperature on germination. His method is to compare the amounts of growth in a given time at different temperatures. The time is measured from the end of the soaking, and in *Pisum* the time-interval chosen is forty-eight hours. For *Pisum* he concludes that the temperature at which germination proceeds most quickly is below 22° . This method seems to be defective, since the amount of growth of the roots in the rather long period of forty-eight hours will be much affected by the ease or difficulty with which the root bursts the seed-coat, there being great differences in this respect between peas treated in exactly the same manner and giving the same growth-curve afterwards.

Köppen ('70) finds that alterations of temperature exercise a retarding influence on growth, and makes a number of determinations of the rates of growth at temperatures, for *Pisum*, from 10° to 40° . These values show a great inconstancy, and besides, in view of the experimentation-time of forty-eight hours and the method employed, their value is small.

Petersen ('74) points out the obvious defects of Köppen's work, demonstrates that variations in temperature, as such, have no effect upon growth, and that the curve of growth at non-injurious temperatures must be a curve convex to the temperature-axis.

Sachs ('87) investigates the occurrence of the Grand Period in different seedlings, and finds it to occur in *Pisum* on the ninth or tenth day. It is to be noted that in his experiments on the fifth to sixth day, the temperature varies between 10° and 19.8° , a variation sufficient to disturb the result considerably. He gives figures for the relation of growth to temperature in *Zea Mays*, and quotes a judicious selection from Köppen's figures showing a relation agreeing in type with his own determinations.

Askenasy ('90) finds for *Zea Mais* that the Grand Period curve is very flat; the maximum rate of growth is reached when the roots are 30 to 40 mm. long, and the rate remains constant till the appearance of the side-roots when the main roots are about 130 mm. long. He finds also, that in roots grown at a high temperature and suddenly subjected to a temperature of 3° to 6° the growth is suddenly stopped (contraction usually occurs), and that, on the return of the roots to the initial temperature, the rate is depressed below the normal rate, for a time depending on the lowness of the intermediate temperature, and the length of their subjection to it.

True ('95) confirms these latter experiments of Askenasy, using *Vicia Faba*, *Lupinus albus*, and *Pisum sativum*, and finds further that a sudden change from a low to a high temperature is, if the interval be great enough (from 3° to 18°), followed by a sudden elongation of the root, with a subsequent period of depressed growth. His experiments with *Vicia Faba* on the effect of transitions between 18° and 30° are of very doubtful value, since 30° is, for *Vicia*, 'above the optimum'.

More recently Schmidt ('13) has demonstrated for *Humulus Lupulus* the very close dependence of growth, under ordinary open-air conditions, upon temperature. His growth and temperature curves vary, in all cases, in the same manner, and often (cf. No. 17) almost exactly proportionally.

Finally, Vogt (cited in Jost, '13) gives a series of determinations, but here nothing is told of the method of experimentation, and the experimentation-time is again 24 hours, a time, as will appear later, much too long, at least for high temperatures.

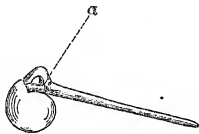
The experiments to be described are on *Pisum sativum*, the material belonging to one sample, carefully mixed at the beginning; and they are in two parts. The first part consists of experiments with the long experimentation-time of 22½ hours. Their purpose was, first, to map out the field, and second, by the use of much larger numbers than is possible in more accurate microscopic work, to afford an idea of the amount of variation in the material. In the second part, the experimentation-time is short, and the determinations made by microscope measurements.

First Series.

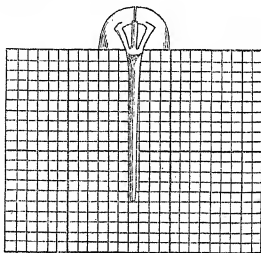
The time occupied daily in preparing material and making measurements in the first series of experiments being about one and a half hours, the experimentation-time became conveniently 22½ hours. At first (the experiments were begun in December), the peas were soaked and germinated at room temperature, about 15° C. during the day. But soon it was found that the temperature fell too low during the night to give a suitable length of root on the third day, and from then onwards the soaking and germination took place in an electric thermostat whose temperature varied slowly between 15° and 17° C. The peas germinated in an apparatus first

constructed by Professor Johannsen and not as yet described. A photograph of it appears in Plate I, Fig. 1, and the apparatus is made in the following manner: A dish of suitable size is chosen and fitted in the foot with a plate of cork. The whole is lined with paraffin, and pieces of wire, or needles, are stuck in the cork in pairs, at such distances that two rubber tubes, which are placed round them, press closely together. The dish is filled with plaster of Paris, and when it has set the rubber tubes are pulled out, the form removed, and the apparatus is ready. A lid as shown in the photograph is quite simply made with a similar lined dish and a smaller plate of cork, covered with paraffin, to press into the soft plaster of Paris. Convenient sizes for the germination of peas are: whole apparatus, 10×15 cm., and rubber tubes 0.7 and 0.5 cm. in diameter, 0.6 cm. apart.

For use the apparatus is thoroughly moistened and is set in a dish containing a little water. In the experiments proper, as distinct from the first



TEXT-FIG. 1.



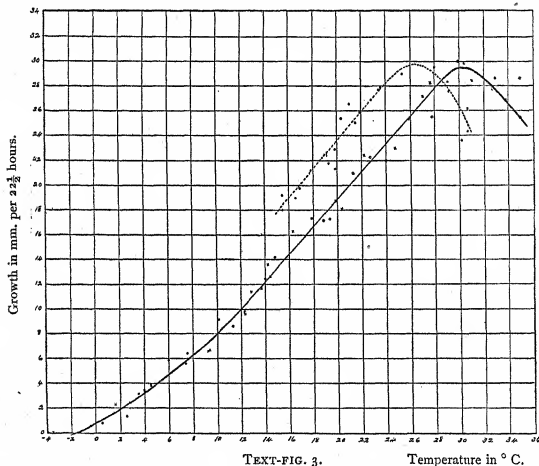
TEXT-FIG. 2.

two days' growth, instead of the porous plaster-of-Paris lid, a bell-jar lined with filter-paper was used to cover the peas, and a petri dish with NaOH was placed above the peas to prevent the accumulation of CO_2 .

The peas were soaked for $22\frac{1}{2}$ hours, and then placed seventy altogether, each day, on this apparatus and left for $46\frac{1}{2}$ hours. At the beginning of the third day after soaking, therefore, the thirty-five most uniform in length were selected and measured. The method of measurement was as follows: the triangular piece of the seed-coat burst up in germination was removed, and a piece of millimetre-paper placed behind the root so that a centimetre line came exactly behind the point marked *a* in Text-fig. 1. The length of the root was read off in millimetres, so that Professor Johannsen's method of measuring 'up to' was used; that is, the next higher millimetre was always taken as the reading. In Text-fig. 2, which shows the paper in place, the length is 15 mm., but, if the

root-tip had been between the 14 and 15 mm. lines, it would also be called 15 mm.

The experiments were carried out in a gas thermostat, where it was possible to vary the temperature from 11° upwards. The temperature was recorded by thermograph tracings, and even at the highest temperatures used the fluctuations shown did not exceed 0.5° . Temperatures below 11° were obtained according to the weather in a room of very constant temperature, where a thermograph tracing was a straight line for days if the windows were undisturbed and the door kept closed. Temperatures near to and below zero were obtained in the open air. The lowest was in an experiment where the temperature did not rise above -2° and where the minimum



TEXT-FIG. 3.

Temperature in ° C.

was -5° . Here no growth took place; instead, a slight general contraction. The roots were frozen, but, on thawing at room temperature, continued to grow.

It was intended that the experiments should be in two series; the first was completed, and in the second—which began about 15° —for some cause which I have been quite unable to explain, the values of the growth-rate suddenly rose and maintained a level uniformly above those of the first series to 28° , after which the usual fall of the rate took place, that is to say, at a temperature which is about two degrees lower than in the first series. At this point, in view of these results, the number of peas soaked each day

was reduced to the minimum possible. About ninety were required, since about 20 per cent. are injured or deformed or of extremely large or small size. It was thus hoped to eliminate unconscious selection of the soaked peas. From then onwards, too, the germinating apparatus and all dishes used were periodically sterilized as a precautionary measure. Under these conditions a third series was carried out, and it agreed exactly with the first.

Detailed figures are given in Table I, and the results are plotted in Text-fig. 3. Table I shows the average initial length, average growth in $22\frac{1}{2}$ hours, together with the standard deviation and probable error of the mean for each experiment. The experiments are arranged in chronological order.

Second Series.

In the second part, the peas were grown in the following way. They were soaked and germinated as before in the electric thermostat, but remained in the plaster-of-Paris apparatus for only one day, at the end of which the roots were about 5 to 10 mm. long. Then they were placed in tubes measuring 1.8 cm. in diameter and 10 cm. in height, with corks bored eccentrically to admit the thermometer during the experiment, and having a triangular piece cut out at one side so that the air in the tube was in contact with the outer air at all times. In the cork a capillary glass tube was fixed so that it lay along the side of the tube, and the pea was fixed to the cork by a pin so that the root was in contact with the side of the tube, between it and a slip of filter-paper and just beside the capillary tube. The filter-paper dipped in water. The root was thus supplied with sufficient moisture at all temperatures and air had free access to it. It was found that without the capillary tube the filter-paper adhered to the glass, and the resistance thus offered to the growth of the root was sufficient to cause curvatures. Plate I, Fig. 2, is a photograph of peas so grown. By this method a large proportion of the peas grew diagrammatically straight, and only a small proportion were incapable of accurate measurement. A slight curvature, such as that shown in the tube to the right in the photograph, is of no significance, since the experimentation-time is so short that the inclination does not appreciably change. In these cases, readings are taken with the eye-piece slightly rotated so that the micrometer scale is perpendicular to the axis of the root.

With the magnification used, a reading 1 on the micrometer scale represented 0.056 mm.

A beaker served as a water-bath, and the temperature was regulated by a micro-burner and Roux's regulator. Except at low temperatures, the temperature of the water remained constant to within 0.5° . For temperatures below 15° a slow current of water flowed through the beaker; a conical

flask was interposed between the beaker and the tap, and for temperatures between that of the water and 15° the micro-burner was used to heat the flask. Between 1° and 5° ice was used. By these means a complete range of temperatures from 1° upwards was available.

The experiments from 15° upwards were performed on a table built into the ground and therefore free from any danger of disturbance by vibration or shaking. At lower temperatures they were carried out in a colder room where such an arrangement was not available, but the room was a basement one, and repeated tests showed no disturbance by shaking.

In view of the great amount of trouble to be saved by conducting the microscope experiments in daylight, a series of experiments was made to test for a possible influence of a sudden transition from dark to light, or as Vogt ('14) incidentally records for *Lupinus*, in his paper on the influence of light on growth in *Avena*, for a possible increase of the rate of growth in light. The temperature of about 25° was chosen for these experiments as giving a high rate of growth likely to show reactions clearly, and as being subject to no injurious effect.

Table A gives the results of these experiments; the readings are half-hour readings, and the numbers micrometer divisions. In Experiments 3, 7, and 9, a 100-C.P. electric lamp at about fourteen inches was used in addition to daylight.

TABLE A.

↓ Commencement of light. ↑ Stoppage of light.

1. $1.7, \downarrow 1.7, 2.0, \uparrow 1.9, 2.1.$
2. $1.6, \downarrow 1.6, 1.6, \uparrow 1.8.$
3. $1.5, \downarrow 1.5, 1.5, 1.5, \uparrow 1.7, \downarrow 1.7, 1.5, \uparrow 1.5.$
4. $1.7, \downarrow 1.6.$
5. $1.5, \downarrow 1.5.$
6. $1.6, \downarrow 1.7.$
7. $\downarrow 1.4, \uparrow 1.1, 1.4, 1.8.$
8. $1.5, \downarrow 1.5, \uparrow 1.55, \downarrow 1.65.$
9. $1.7, \downarrow 1.5, 1.3.$

The means are, in dark, 1.6 ; in light, 1.55 . These readings show that, neglecting for the moment Experiments 3, 7, and 9, the fluctuations in the readings show no constant relation to the presence or absence of light. The fluctuation is, indeed, such as takes place in experiments under uniform conditions. (Compare Experiments 3 and 4 on change of temperature, quoted below.) Experiment 9 shows a fall in light, and Experiment 7 a rise in darkness after illumination—which results do not necessarily confirm each other—and Experiment 3 under the same conditions shows, as the experiments in daylight alone do, no influence of the light.

To illustrate the absence of any 'stimulatory' effect, Experiment 2 may be cited in full.

		↓							↑						
Time.	2.49,	3.19,	3.25,	3.31,	3.37,	3.43,	3.49,	4.19,	4.49.						
Growth.	1.6	0.3	0.3	0.3	0.3	0.4	1.6	1.8							

The fifth reading in light is due to the inaccuracy in reading, readings being 'up to' one micrometer division (0.056 mm.), and the rise to 1.8 in the last half-hour is a difference such as occurs as a normal fluctuation under uniform conditions.

Two of the experiments to be described later, on the Grand Period of growth, were also performed in darkness for comparison, and they showed no difference from those in light. The shoot was etiolated, but the rate of growth of the root was unaffected. Therefore, at least within the conditions under which all the rest of the experiments took place, light exerts no influence on the rate of growth.

In the next place there is to be considered the effect of a sudden rise or fall of temperature. In all experiments the same results were found, namely that the roots assumed immediately on reaching the new temperature the rate of growth characteristic for it, and that, for temperatures up to 29°, such fluctuations as take place afterwards occur without showing either a typical time or mode of occurrence, so that, taking the mean of a number of peas, the rate of growth, for at least six hours, is represented by a straight line. To illustrate this the following experiments are quoted:

1. *Fall to low temperature from thermostat temperature.*

Date.	Time.	Growth.	Temperature.
March 7.	2.27 p.m. to 2.57 p.m.	0.1	4.9°
	2.57 p.m. to 3.27 p.m.	0.1	—
March 8.	11.42 a.m. to 12.12 p.m.	0.1	4.5°
	12.12 p.m. to 12.42 p.m.	0.1	4.5°
	12.42 p.m. to 1.12 p.m.	0.1	4.5°

2.

March 7.	3.30 p.m. to 4 p.m.	0.1	4.9°
	4 p.m. to 4.30 p.m.	0.2	4.9°
	4.30 p.m. to 5 p.m.	0.2	4.9°
March 8.	5 p.m. to 10.27 a.m.	0.11 (mean)	3.5°
	10.40 a.m. to 11.10 a.m.	0.1	3.5°
	11.10 a.m. to 11.40 a.m.	0.2	3.9°

The fall here is from thermostat temperature, 15° to 17°, to about 4°. In both, the characteristic low rate of growth is assumed at once and maintained. In No. 2, where the growth during the night was measured, giving a mean rate of 0.11, a fall is seen as compared with the rate from 3.30 to 5, which is 0.17, due to the fall of temperature from 4.9° to 3.5° during the night. In No. 1 the rate on the 8th is the same as on the previous day, it being measured at nearly the same temperature.

3. *Fall from high temperature to room temperature.*

Rate of growth given first for three half-hours, then in six-minute readings :

Temperature.	27°, 27°, 27°, 27°, 25.5°, 21.2°, 20.3°, 20.2°, 20.0°, 19.8° = room temperature.
Rate of growth.	1.8 1.8 2.1 0.2 0.2 0.3 0.2 0.2

4. *Rise of temperature.*

Rate of growth given during first half-hour in six-minute readings, then in half-hour readings :

Temperature.	20.0°, 25.0°, 25.8°, 26.0°, 26.0°, 26.0°, 26.1°, 26.0°
Rate of growth.	0.3 0.4 0.3 0.3 0.3 1.6 1.8

5.

Temperature.	23.8°, 26.5°, 27.0°, 27.0°, 27.0°, 27.0°
Rate of growth.	0.3 0.3 0.3 0.2 0.3

6.

Temperature.	23.9°, 26.1°, 26.5°, 26.6°, 26.6°, 26.6°
Rate of growth.	0.4 0.3 0.3 0.4 0.3

7. Finally the following experiment, where the results given are the means of measurements of eight peas: the rise of temperature is from room-temperature, about 18° to 25.2°.

In the first, second, and third interval of ten minutes, the mean rate of growth was: 0.50, 0.475, 0.475; that is, practically identical; and the rates in the first and sixth half-hours were 1.45 and 1.45.

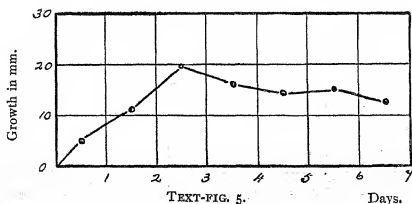
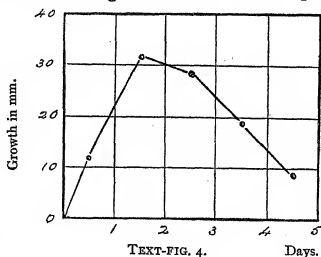
From these experiments it will be clear that a change of temperature is, in itself, without effect; but that the rate of growth follows immediately and accurately any considerable change of temperature.

The next point to be considered is the possible effect on the experiments of the Grand Period of growth of the roots. In view of Sachs's determinations, it appeared at first as if, in my experiments, taking place always at the beginning of the third day of growth, the slight rise to be expected during that day would be negligible, at least in short-period experiments. But in the long-period experiments at higher temperatures it had already become probable to me that Sachs's results did not apply, and therefore, at the beginning of the microscope experiments, a series of determinations on the time of occurrence of the Grand Period was begun and continued intermittently. The roots were grown in long test-tubes with exactly the same other arrangements as in the microscope experiments, and measurements were made by applying a millimetre scale to the outside of the tube, a method which avoids all disturbances of growth by handling. Table II gives a full account of the results. Measurements were never continued after the appearance of the side-roots.

The conclusions from Table II are (1) that the roots germinated and grown at a constant temperature (Expts. 9, 10, 11) show the Grand Period on the third day at 14° C., and on the second day at 23° C.; (2) that roots germinated and grown for one day at a temperature of 15° to 17° C. and then transferred to a different constant temperature show the Grand Period also on the third day, but if they be transferred to a different higher temperature at the beginning of the second day, the Grand Period occurs on the second day. Thereafter, in all cases, the rate falls slowly to the time of appearance of the side-roots; (3) that the side-roots appear at a definite length of the main root—in all cases except one, when the main root is between 80 and 90 mm. long. They therefore appear earlier, in time, at high temperatures than at low. This agrees with Askenasy's results so far as the appearance of the side-roots at a particular length of the main root is concerned, but he found that the rate of growth of the main root maintained a constant value from the beginning of the Grand Period till then.

Text-figs. 4 and 5 show the results of Expts. 9 and 10 respectively.

With regard to the bearing of these facts on the experiments in general,



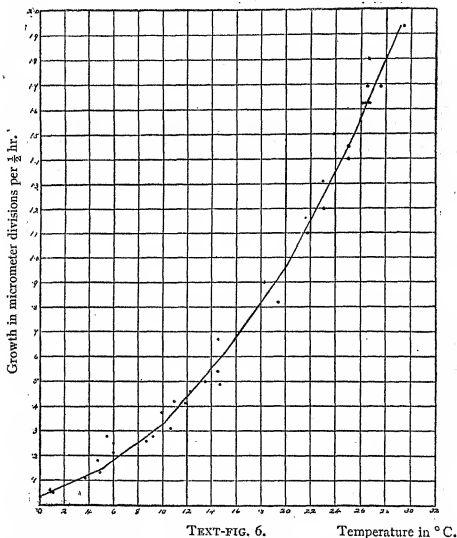
all measurements, being made at the beginning of the third day of growth, are made in that phase where the rate of growth is, as nearly as possible, at its highest value and most constant. Further, as has already been deter-

mined, the rate of growth, under the conditions of experimentation, is actually constant during the whole of the third day at very low temperatures and at 25° for at least six hours. Since, therefore, all points on the curve of growth are means of readings taken over usually three half-hours (sometimes one or two; never more than six), it may be taken that the difference between readings at different temperatures is entirely due to the difference between the temperatures. On the other hand, in the long-period experiments there will probably be a slight disturbance due to the passing of the Grand Period during the time of the experiment.

At the commencement of the microscope experiments, I decided that most time should be devoted to low temperatures and to high, because, on the one hand, I thought that there might be a possible inaccuracy in the long-period determinations at low temperatures due to the length of time necessary for the apparatus to sink from the temperature of the laboratory, at which measurements were made, to the temperature of the experimentation room or the open air; and, on the other hand, the long-period method is impossible as a means of accurate analysis at high temperatures. The number of experiments at low and at high temperatures is therefore much greater than at medium temperatures, but the agreement of the determinations at medium temperatures shows them to be sufficient. At low temperatures it was found that the new results agreed with the long-period experiments: but already at about 10° the two curves diverge, and separate more and more rapidly to 29° . An analysis of the reasons for this has not been possible, but two things are probable, (1) that the Grand Period affects the long-period experiments to some extent, and (2) that the difference in the conditions of experimentation accounts for a considerable part of the difference. At least at temperatures above 20° , preliminary experiments, before the method described for the microscope experiments was determined upon, showed that the question of sufficient moisture is of the greatest importance, and that devices such as lining the tube with moist filter-paper (perhaps comparable to, though not so good as, the conditions in the long-period experiments) were quite insufficient to supply the roots with the necessary moisture. With the more perfect water-supply a higher rate of growth is to be expected, and was found.

The experiments from 0° to 20° were in one series, those from 20° to 28° in two series, and for higher temperatures the experiments were in several series. This precaution was adopted to prevent any possibility of disturbance through the operation of a particular set of conditions, as occurred in the experiments of Series I. No indication of any such appeared; control experiments, repeated during the experiments at high temperatures to make sure of the good condition of the roots, always gave results agreeing exactly. The experiment at 25° quoted in connexion with the question of 'temporary stimulation' by a rise of temperature (Expt.

No. 7) was the last experiment done, and it shows conclusively that no alteration or disturbance in the material had occurred, for the value it gives lies exactly on the growth-rate temperature curve. Table III gives the results from 0° to 29°, and Text-fig. 6 shows the relation of growth to temperature in graphical form. Up to and including 28°, the growth-rate is constant during the time of the experiment.

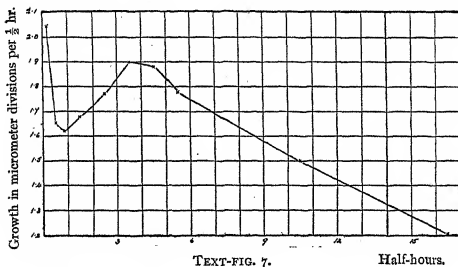


At temperatures between 28° and 30° a new factor comes into operation. At this point, experimentation becomes very difficult. On raising the temperature to about 29°, in most cases rapid and sharp curvatures took place. These curvatures are not the gentle curvatures already referred to, extending over perhaps half the length of the root and altering in direction so little and so slowly as to have no effect on the accuracy of the measurements. These occur over only about 5 mm. at the tip of the root and result in a sharp inward bend of the tip, which makes measurement impossible. More than one in two of the roots used curved in this way so that they could not be measured at all, and of the others, most could be measured only for two half-hours. Any number of measurements, however great at

this temperature, would therefore be an analysis of less than half the material. Indeed, this fact, together with the fact that above 30° a few similar curvatures still take place, shows that this point is a critical point at which, even if measurements in numbers were easy, an accurate analysis would be made very difficult, if not impossible, by the necessity for referring each individual to one or other of two types, according to whether a time-factor is or is not operating, as will be clear later.

Above 30° measurements are again easy; a few curvatures still occur, but the proportion is small enough to be negligible—about 1 in 15.

From 30° onwards, an entirely new set of phenomena appears. I had expected that here it would be possible to get by short-time readings values above the readings at 29° , and that a time-factor would be found operating in accordance with Blackman's theory. A time-factor does operate, but the relation between the rate of growth and the time is not a simple one. Here,



TEXT-FIG. 7.

Half-hours.

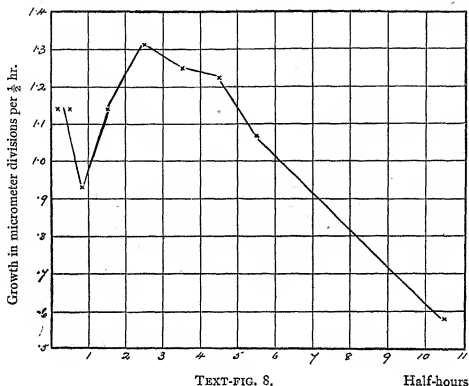
owing to the difficulty of the analysis and the necessity for many experiments, I considered it best not to experiment at random temperature intervals as before, but to restrict the experiments to a few temperatures. They are, approximately, 30° , 35° , 40° , 42.5° , and 45° , and Table IV gives the results for these temperatures. The results for 30° , 35° , and 40° respectively are plotted in Text-figs. 7, 8, and 9.

For the experiments at 30° the standard deviation and probable error have been calculated for the readings in the first ten minutes, and for those in the first half-hour. They are:

	Mean.	Standard Deviation.	Probable Error.	No.
First ten minutes.	0.68	0.128	0.033	15
First half-hour.	1.74	0.279	0.043	43

In both cases the probable error is, considering the small number of readings, satisfactorily small, and compares very well with the results in the earlier experiments.

The meaning of the results may be summarized thus: The rate of growth in the first ten minutes at $3\cdot30^{\circ}$ is the highest rate obtained; in the



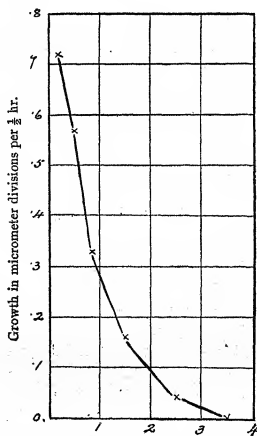
TEXT-FIG. 8.

Half-hours

first half-hour it falls rapidly to a minimum, and thereafter a recovery takes place, giving a second maximum in the fourth half-hour. After that the rate falls uniformly and rather slowly, being about two-thirds of its initial value after eight hours.

At 35° the course of events is similar, but the rise and fall are steeper. Here readings were taken in the five minutes which the temperature takes to rise from about 30° to 35° . These gave a mean value of 0.33 in five minutes, or 0.66 in ten minutes, in contrast to 0.38 in the next ten minutes. It is to be noted that this is nearly equal to, but lower than, that in the first ten minutes at 30° .

At 40° things are again entirely different: here the rate falls uniformly and rapidly, showing no recovery. Here also readings were taken in the five-minute



TEXT-FIG. 9. Half-hours.

period while the temperature rises from about 33° to 40° , and they give a value of 0.50 in ten minutes, which is more than twice as high as the value in the first ten minutes at 40° .

At 42.7° the fall is similar but steeper. At temperatures between 44.5° and 45° , growth can no longer take place. A slight increase in length takes place during the raising of the temperature, but in no case is there any after the temperature has reached 44.5° , and usually contraction occurs immediately. To quote an illustrative experiment :

Readings at one-minute intervals for ten minutes ; then two readings, one after five minutes and one after thirty more minutes :

Temperature.	39°	41.8°	42.8°	43.8°	44.1°	44.3°	44.5°	44.8°	44.8°	44.8°	44.8°	44.9°	44.9°
Growth.	0.1	0	0	0	0	-0.1	0	0	0	0	-0.1	-0.2	-0.5

In this time, forty-five minutes, the root had contracted 0.45 mm., had, as was typical, become discoloured and flaccid and, left at room temperature for two days, showed no sign of recovery. The death-point appears therefore to lie below 45° .

It must be borne in mind however that, with regard to this, the death-point itself is dependent on time. All roots after a shorter or longer exposure to temperatures above 30° became flaccid and discoloured, the normal colour of the root changing to a dull white, and the greeny-yellow of the root-tip to a dull brown. At 35° this required several hours ; but, while all roots left overnight at 35° were killed, the shoots recommenced to grow at room temperature and continued quite vigorously during the next two days. At 40° growth had stopped in most roots after an hour, and in none did it continue after one and a half hours. In two hours the roots were flaccid and discoloured, and neither the roots nor the shoots showed recovery in the next two days. At 42.7° growth occurred only in the first half-hour, and after one hour there was no recovery. At or below 45° , death seemed to be instantaneous.

DISCUSSION OF RESULTS.

Considering the results first from the point of view of Blackman's theory, it appears at once that no extrapolation according to Blackman's method is possible. Even if there were not the sudden drop and recovery at 30° and 35° , the rate during the raising of the temperature from 30° to 35° shows that the rate at 35° never does rise above that in the first ten minutes at 30° . It is even more evident that the rate at 40° never does exceed that at 30° or 35° . Again, the coefficients for a rise of temperature of ten degrees are : $10/0 = 8.25$, $15/5 = 4.07$, $20/10 = 2.90$, $25/15 = 2.38$, $29/19 =$ a value probably greater than 2. They show a very distinct fall as the temperature rises ; only between 10° and 29° do they lie between

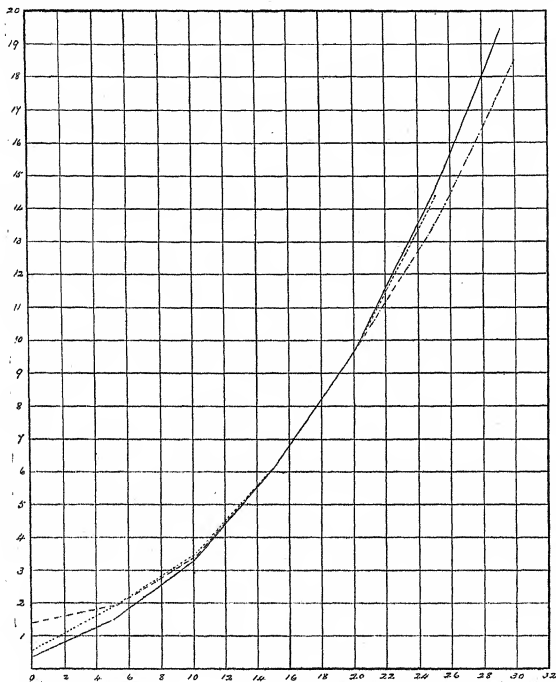
2 and 3. The curve representing the relation of growth to temperature cannot, therefore, be regarded as a van't Hoff curve.

Regarding this point, Pütter, basing his theory on experiments on skin-respiration in the Frog, has attempted to show that the relation between temperature and any life-process may be expressed graphically as a van't Hoff curve, and that deviations from this curve are due to the superposition of exponentials. His experiments are however open to objection on the ground that he neglects to take into consideration the effect of muscle-tone, a point shown by Krogh to be of fundamental importance. Kuijper has worked out the relation between temperature and respiration in *Lupinus*, *Pisum*, and *Vicia*, and regards the curve which expresses the relation as a van't Hoff. In this case, however, as in others cited by Pütter, only the coefficients of the middle region show an approximation to the proper magnitude—between 2 and 3—and this region is arbitrarily chosen as that on which most reliance is to be placed. That there is no question of a middle region of 'correct' coefficients giving place, at the two ends, to regions in which deviations are due to extreme conditions, is indicated by the fact that, in all cases, the coefficients fall continuously from the lowest to the highest temperatures. On the other hand, Krogh insists that for 'standard metabolism' in animals, the curve relating temperature to intensity is not a van't Hoff curve. If we plot my curve with Kuijper's for respiration in *Pisum*, and Krogh's for 'standard metabolism', on the same axes, we find that the three are strikingly similar (Text-fig. 10); and this fact strongly supports the view enunciated by Krogh 'that the temperature relations of physiological processes do follow certain typical curves which seem to be identical or nearly related for processes of the same fundamental nature in different organisms.'

It is interesting, also, to compare Kuijper's results at high temperatures with mine. At 30° and 35° he finds a fluctuating rate of respiration, and at 40° and above, a uniform fall in succeeding time-intervals, also without any possibility of extrapolation by Blackman's method.

The question of terminology may be referred to here. These growth experiments at once suggest the old terminology, minimum, maximum, and optimum. But the use of the word 'optimum' has, since Blackman's ('05) paper on 'Optima and Limiting Factors', fallen, if not into disuse, at least into disrepute. Now the process of growth shows in its relation to temperature three well-marked points. — 2° C. is the lowest temperature at which growth takes place. Between 44° and 45° lies a point above which growth ceases practically instantaneously. About 29° lies a point such that any increase of temperature means the introduction of a time-factor and the consequent continuous decrease in the growth-rate. The same three points are distinguishable in any of the other processes the relation of which to temperature has been studied. The first of these points is the *minimum* ;

the second the *maximum*. It is suggested that the term *optimum* should properly be applied to the third. The reason for the confusion which exists with regard to this term is that the point is usually lower than that



TEXT-FIG. 10.

1, for growth curve, represents growth of 0.1 on Text-fig. 6. Temperature in ° C.

1, for Krogh's curve, represents 39.34 on his scale.

1, for Kuijper's curve, represents 3.03 " "

— Growth.

- - - - - Krogh.

- · - · - · Kuijper.

at which a process shows its maximum intensity, and that the idea of optimum, originally founded on long-period experiments, has been associated with apparent maximum intensity. More exact analysis has shown the

presence of a time-factor, and a change in the conception of the meaning of the *optimum* has become necessary. It is absurd to apply the term, as Kuijper does, to a temperature at which the process shows a very high initial intensity and then a more or less rapid drop to an intensity below that obtained at lower temperatures. It seems logical, on the other hand, to apply it to the highest temperature at which the process takes place at a constant rate. What is important is, that this point possesses an objective reality just as do the maximum and minimum points.

If the term 'optimum' is used in this sense, it becomes necessary to distinguish another point at which the intensity of the process is at its maximum. This may be illustrated by reference to the relation of temperature to respiration in *Pisum*. Kuijper, by using very short experimentation-times, finds that the initial intensity of respiration is higher with rising temperature almost to the maximum temperature. Probably with infinitely short periods it would be found that at the maximum temperature the initial intensity would be the maximum intensity for a very short time. That is, the maximum-rate temperature would coincide with the maximum temperature. In the case of growth, the relation is different. The highest measured rate occurs in the first ten minutes at 30.3° , and it has been shown that, at higher temperatures, a higher rate does not occur even for a very short time. Between 28° and 30° lies the optimum point. Its exact determination experimentally is a matter of very great difficulty, if it is, in fact, at all possible. But it seems probable that, if exact analysis of this interval could be carried out, there would be found a temperature at which the rate would be equal to, or higher than, that at 30.3° and at which no time-factor would be in operation. This would be the optimum, and, in this case, the maximum-rate temperature would ultimately coincide with the optimum. The probable coincidence, in the limiting case of the maximum-rate temperature, with the optimum or the maximum cannot be demonstrated, on the one hand, because of the curvatures which take place, and on the other, because it is impossible to experiment with infinitely short periods. Therefore the maximum-rate temperature remains, at least experimentally, distinct.

It would seem, then, that four cardinal points are to be distinguished for all investigated physiological processes:

The minimum temperature for any process is the lowest temperature at which the process takes place.

The maximum temperature is the highest temperature at which the process takes place.

The optimum temperature is the highest temperature at which there is no time-factor operating, and

The maximum-rate temperature is that temperature at which the process attains to its highest intensity.

With regard to a possible explanation of the second maxima shown in the time-growth curves, at 30° and 35° , reference may be made to a recent paper of Miss Sophia Eckerson ('14) on Thermotropism in Roots. The author shows that changes in the thermotropic reaction are due to changes in the permeability of the roots at certain critical temperatures. In the case of *Pisum*, a decrease in permeability takes place between 30° and 40° , resulting in a negative curvature in roots exposed to a higher temperature on one side, in that range. It is possible that roots exposed to temperatures within that range might show a secondary elongation due to osmotic phenomena, which, superimposed on a simple time-growth curve, would give rise to a temporary second maximum. Too much stress should not, however, be laid on this coincidence. As already stated, Kuijper's respiration determinations also show fluctuations at temperatures between 30° and 40° , and it is possible that both these and the second maximum in growth may be due to deeper, unanalysed metabolic processes.

SUMMARY.

The relation of growth to temperature can be expressed as a uniform curve from -2° to about 29° . It is not a van't Hoff curve, but shows a very close resemblance to the curves found by Krogh for 'standard metabolism in animals', and by Kuijper for respiration in *Pisum*.

Above 29° the relation can no longer be expressed as a curve, but for each higher temperature a different curve must be constructed to express the rate of growth in successive time-intervals. Between 30° and 40° these curves are not simple time-curves, and no extrapolation is possible.

For growth there is a well-marked optimum temperature. The general applicability of this term to physiological processes is considered, and a definition offered. A further point, the maximum-rate temperature is distinguished and defined.

For growth, the minimum temperature is -2° ; the maximum 44.5° ; to the degree of accuracy found possible the optimum temperature is between 28° and 30° , and the maximum-rate temperature is 30.3° .

This work was carried out in the University Plant-physiology Laboratory in Copenhagen during my tenure of a Carnegie Research Scholarship. I have to express my indebtedness to Professor Johannsen for the suggestion to perform these experiments, and for the opportunity to do so; and to Dr. Boysen-Jensen for many helpful suggestions, for his continued interest in the work, and for much of that sceptical criticism which keeps one in the strict path of verification.

TABLE I.

In each experiment thirty-five peas were used ; the figures for initial length and growth are means. The columns give, in order, initial length, growth in mm. in 22½ hours, temperature, standard deviation, and probable error of the mean.

The experiments are in chronological order.

<i>Initial Length.</i>	<i>Growth.</i>	<i>Temperature.</i>	<i>Standard Deviation.</i>	<i>Probable Error.</i>
Series I.				
18.91	8.56	11.4°	1.7354	0.2976
21.23	14.18	14.8°	2.4428	0.4252
22.79	17.32	17.8°	2.7911	0.5275
21.74	17.09	18.8°	3.9511	0.6776
21.18	17.32	19.3°	3.0308	0.5443
14.17	21.00	21.3°	2.9186	0.5727
14.74	22.33	22.7°	2.8922	0.5047
16.96	22.00	24.0°	3.8829	0.7615
21.13	25.33	25.8°	3.8588	0.7045
20.80	25.45	27.8°	4.0797	0.7102
19.68	30.00	29.9°	5.3742	0.9217
15.97	28.60	32.9°	5.5519	0.9521
14.65	25.35	34.9°	4.9254	0.8447
Series 2.				
16.27	5.89	6.0°	1.2598	0.2129
15.82	6.62	7.5°	1.2133	0.2081
18.94	14.44	14.0°	1.8179	0.3118
19.53	19.17	15.5°	1.6302	0.2756
19.38	18.03	15.5°	2.3288	0.4054
19.96	19.00	16.5°	2.5071	0.4238
21.65	19.76	16.8°	3.2568	0.5669
18.44	21.18	17.8°	2.3695	0.4064
17.72	21.93	19.3°	2.1763	0.4188
18.40	21.37	19.8°	3.5955	0.6564
13.63	22.90	19.8°	3.6534	0.7618
11.24	25.18	20.3°	2.9566	0.4998
10.47	26.29	21.0°	3.2970	0.6019
12.53	25.02	21.5°	2.8113	0.4752
14.65	25.94	21.9°	3.1660	0.5338
15.71	27.68	23.3°	2.9061	0.5059
14.56	27.86	23.5°	3.2195	0.5521
12.24	28.97	25.4°	3.2897	0.5561
10.85	32.76	27.7°	2.7343	0.5362
9.26	29.46	28.0°	1.6416	0.3981
20.75	26.08	30.7°	3.9176	0.8760
19.50	24.28	30.7°	4.4981	1.3562
16.5	23.56	30.2°	3.2528	0.5939
10.76	27.64	29.0°	2.1393	0.3842
15.57	1.40	2.5°	0.8000	0.2066
9.77	0.82	0.25°	0.3856	0.1162
10.87	28.18	29.0°	2.7500	0.6875
9.15	0.68	2.75°	0.4583	0.1025
Series 3.				
11.80	3.17	3.5°	0.9545	0.1662
14.13	3.77	4.5°	1.0993	0.1858
12.65	1.20	2.5°	0.4679	0.0802
12.09	5.61	7.3°	1.1709	0.2008
13.39	9.83	12.3°	1.8377	0.3106
14.50	9.14	10.5°	1.3717	0.2319
13.79	6.72	9.3°	1.1097	0.1903
11.14	6.56	9.2°	1.2338	0.2148
10.67	9.57	12.3°	1.3896	0.2580
12.24	11.35	12.8°	1.4783	0.2535
13.88	11.64	13.6°	1.8935	0.3242

TABLE I (continued).

<i>Initial Length.</i>	<i>Growth.</i>	<i>Temperature.</i>	<i>Standard Deviation.</i>	<i>Probable Error.</i>
14.33	13.61	14.1°	1.5340	0.2849
14.89	12.65	14.4°	2.3581	0.4105
13.21	16.27	16.3°	2.4438	0.4389
16.15	18.84	19.9°	2.2701	0.4452
23.63	18.13	20.3°	3.8966	1.0061
20.43	22.49	22.2°	2.2871	0.4402
20.54	22.96	24.7°	3.7125	0.7145
15.97	27.20	27.0°	3.5358	0.6064
14.97	28.26	27.7°	5.0273	0.8622
13.16	29.89	30.3°	2.5092	0.4436
13.79	28.37	31.0°	4.7522	0.8150
12.53	26.93	33.8°	4.2702	0.7548
	0.0	-3.5°		
14.35	0.56	-0.5°	0.2449	0.0837
14.39	2.38	2.8°	0.8400	0.1420
12.53	3.36	4.0°	0.6001	0.1029
14.47	3.87	4.5°	1.0738	0.1869
13.44	2.31	1.5°	0.7079	0.1197

TABLE II.

Grand Period Experiments.

The columns give in order (1) the number of the experiment; (2) the number of days of growth before the change of temperature from thermostat temperature; (3) the temperature of the experiment; (4) the day on which the Grand Period occurs; (5) the growth in mm. on that day; (6) the day on which the side-roots appear; (7) the length of the main root, in mm., at the end of that day; (8) the number of peas measured in each experiment:

1	2	3	4	5	6	7	8
1.	2	10°-12°	3	13.7	10	84.5	3
2.	—	15°-17°	3-4	14.5	10	81	2
3.	2	16°	3	18	6	85	1
4.	2	25°	3	33	4	87	1
5.	2	25°	3	27	4	71	1
6.	1	26.2°	2	38	3	81	1
7.	2	26.2°	3	35	4	83	1
8.	2	26.8°	3	38	4	87	1

Conditions uniform from beginning:

9.	—	23°	2	31.5	4	85	6
10.	—	14°	3	19.8	7	87	5
11.	—	14°	3	19.0	7 and 8	89	5

In Experiment 2 there is no change of temperature; it is at thermostat temperature throughout.

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Since this paper has been in course of publication a report has appeared in Botanisches Centralblatt (1915, No. 51, p. 662) of a paper by P. A. Lehenbauer [Physiol. Res. I, 1914, pp. 247-288] on Growth of Maize Seedlings in relation to Temperature. The paper, however, I have not been able to procure.

For Descriptions of the figures on Plate I, illustrating Miss Leitch's paper, see pp. 27 and 29.

A Description of a Recording Porometer and a Note on Stomatal Behaviour during Wilting.

BY

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AND

R. C. KNIGHT, B.Sc.¹

With three Figures in the Text.

IN experiments involving the estimation of stomatal aperture it is often necessary to obtain a continuous record of stomatal behaviour over a considerable period. The necessity for making continuous observations by any of the usual methods is inconvenient, especially when observations of other phenomena have to be made. Balls (1) has used with success an automatic recording porometer, the Stomatograph, the chief objection to which is its high cost. More recently, Neilson Jones (2) has devised a recording porometer which can be constructed in the laboratory from inexpensive material. The apparatus here described is also of simple construction, and it has been found very satisfactory for stomatal investigations.

The apparatus is essentially a self-recording modification of the aspirator porometer which has been described by one of us in a previous paper (3). In that paper it should have been stated that Balls (1, p. 34) has experimented with a recording aspirator, which, however, was subsequently abandoned for the type of apparatus of his Stomatograph.

In the present apparatus a head of water in a constant-pressure aspirator is employed to draw air through the leaf, and the speed of the air-stream (and therefore the relative size of the stomatal apertures) is measured by the rate at which water flows from the aspirator.

The apparatus is shown diagrammatically in Fig. 1. A is a wide-mouthed bottle fitted with a rubber stopper pierced by three holes, through

¹ This work was undertaken by both authors jointly, but owing to the death of Mr. Laidlaw, who fell at Richebourg l'Avouée in April, 1915, the second author is alone responsible for the statements in the paper.

which pass three glass tubes. Tube B is connected with the leaf-chamber attached to the plant, and passes almost to the bottom of the bottle. The air drawn from the leaf enters the bottle through B. C is the water-exit tube through which water is siphoned from the bottle through tube E, whence it flows drop by drop on to the recorder. The siphon tube C is provided with a three-way stopcock, F, by means of which the aspirator bottle can be refilled from the funnel when necessary, air escaping through the tube and stopcock D during the operation. The head of water used to draw air through the leaf is regulated by the difference in level between the lower

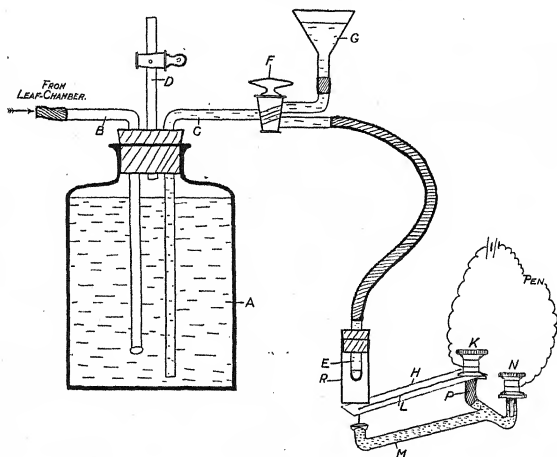


FIG. 1.

ends of tubes B and E. E is connected to the siphon tube, C, by a piece of thick-walled rubber tubing, so that any required pressure difference may easily be obtained by raising or lowering E.

When the rate of dropping is slow, each drop remains at the end of tube E for some time before falling, and there is a danger of its being prematurely removed by air currents. To prevent this, E is surrounded by a wide tube, R, secured by a rubber stopper. The inside of R is kept damp by lining it with wet blotting-paper, in order to reduce evaporation from the drop, which might conceivably be considerable under some conditions.

The speed at which the water flows from E is measured by the frequency of the drops as determined by the recorder. This consists

of a strip of mica, H, secured by a binding-screw, K, at one end, carrying a thin platinum wire, L, which is bent downwards at the free end and poised over mercury in a glass tube, M, which connects with the binding-screw N. The binding-screws are secured in their respective tubes by means of sealing-wax, and K is separated from the mercury by a sealing-wax plug, P. Leads from K and N are carried to a battery and the terminals of a magnetic pen, writing on an ordinary clockwork recording drum. We have used and found satisfactory a drum supplied by the Cambridge Scientific Instrument Company. It is about 13 cm. in diameter and revolves once in about twenty-seven minutes, being long enough to record continuously for forty hours.

The mica strip is placed so that the drops from E impinge upon its free end. Thus, when a drop falls, the platinum point carried by the mica is plunged forcibly into the mercury in M, and by this means a circuit is completed through the battery and pen, and the pen is deflected, making a record on the drum. The resilience of the mica immediately withdraws the platinum point from the mercury and poises it in readiness for the next drop, so that the current passes only momentarily. In order that water may not accumulate on the mica and so prevent the strip from springing back and breaking the contact, it has been found advisable to slope the mica strip so that the water drains from the end. The mercury retains a film or drop of water upon its surface, which, however, is no disadvantage, since even if the platinum continuously dips into the water, the low voltage (about two volts) required to work the pen produces very little electrolysis, and at the same time the effects of sparking, which occurs when the contact is broken, are lessened.

It has been found that the mica strip tends to oscillate after a drop has fallen, thus making more than one record on the drum. This is not a serious drawback because it is readily detected when it occurs; it is, however, easily prevented by raising the recorder till the tube R bears upon the mica, thus pressing the platinum point very close to the mercury surface. This is found to damp the oscillations and one record per drop is obtained.

The chief difficulty with the apparatus is that when air has entered the aspirator bottle, temperature changes cause changes in the volume and a consequent alteration in the rate of dropping. To overcome this, it has been found necessary to place the aspirator in a water bath at a constant temperature. An electrically heated bath, constant to $\pm 0.02^{\circ}$ C., has been used and found extremely satisfactory, but such accuracy as this is not essential. It is obvious that a greater volume of air in the bottle entails less accuracy, so that an experiment should be started with the bottle full of water, and refilling should be resorted to as frequently as possible.

Recently boiled distilled water is used in the aspirator in order to avoid the inconvenient accumulation of air bubbles in the tubes of the apparatus, when the temperature of the water is raised to that of the bath.

A record obtained with this apparatus consists of a series of marks upon the clockwork drum, the distance between two adjacent ones representing the time taken for a drop to be formed and discharged. The distances between adjacent marks will therefore be a measure of the rate at which air is being drawn into, and water out of, the aspirator bottle if the drops are all of the same size. Tests have been carried out to determine the weight of water per drop. Successive single drops were found to vary considerably less than 1 per cent., and this variation includes errors of collecting and weighing. Variation in the rate of dropping produces variation in the size of the drops—a quicker rate giving larger drops, but this variation is most marked with quick rates. Over the range which has been found convenient in experiment, i. e. not quicker than two or three drops per minute, the variation in size of the drops was again less than 1 per cent.

In addition to the change in size of the drops, error may result from a change of temperature of the air in the glass or rubber connexions which are not in the water bath, namely the connexion from the leaf-chamber to the air intake-tube of the aspirator bottle. Such a temperature change would result in a change of volume, and consequently of the rate of dropping. To reduce this error to a minimum it is advisable to reduce the volume of air in these connexions to a minimum, by using narrow-bore thick-walled rubber and glass tubing, and to shorten the distance between plant and aspirator as far as possible. If the whole apparatus is shaded from direct sunlight sudden temperature changes will be avoided. A water jacket for these connexions has been contemplated, but it has not been found necessary to resort to this.

Before employing the apparatus for investigation of stomatal changes, many preliminary experiments were carried out, using fine capillary tubes and platinum discs with small punctures in them, in order to estimate the accuracy of the apparatus under constant conditions. No purpose would be served by a full account of these preliminary investigations, but the conclusions drawn from them will be briefly outlined.

Several types of air intake-tube were tried, the air being made to bubble from apertures of various sizes and shapes, but a square-cut end was found to be as satisfactory as any form. Similarly a square-cut dropping-tube (E in Fig. 1) was found satisfactory.

Slow rates of dropping tend to decrease the accuracy, possibly due to the fact that when a drop remains for some time attached to the dropping-tube there is a greater possibility of its being prematurely shaken off, and also greater opportunity for temperature changes to become effective. The most convenient and at the same time accurate rates are from one drop

per thirty seconds to about one drop per 120 seconds. In work with plants, the rate can be arranged by selection of the leaf-chamber and regulation of the pressure difference in the aspirator. Naturally, owing to stomatal changes, the rate changes considerably during a day, and very slow rates are unavoidable, but for short experiments in daylight the above-mentioned speeds have been found easy to arrange.

The experiments with fine capillaries and punctured platinum discs gave indications of the existence of a rhythm in the flow of air, and although it was realized that some of the observed irregularities might be due to the capillaries themselves, attempts were made both to determine and to eliminate this rhythm. It was thought to be due to the small pressure changes resulting from the relative sizes of the drop at E and the bubble at B, and various combinations of sizes of these two tubes were tried, but failed to give satisfactory results. As the period of the rhythm probably changes with temperature and rate of flow, attempts to calculate it were given up, especially as the later experiments upon stomata gave sufficient accuracy without allowing for a rhythm.

It was evident, however, that the disturbing influence of the rhythm was less noticeable with large than with small pressure differences, so that in so far as is compatible with other conditions of the experiment, it is advisable to employ large pressure differences; from 5 to 15 centimetres have been found convenient.

By taking the precautions outlined above, it has been found possible to obtain very satisfactory results with the apparatus: thus, on July 2, 1914, with the apparatus attached to a fine capillary tube and a pressure difference of 10 cm., a record running for two hours gave an average distance travelled by the drum during the formation of one drop of 5.0 divisions of the paper used (= about 55 sec.), the maximum distance for any drop being 5.2 divisions and the minimum 4.8. Typical records obtained with stomata are given in the second part of this paper. Test experiments have also been carried out to compare the records obtained with the apparatus with that obtained with the ordinary porometer by stop-watch readings, of which the following is typical:

Exp. 110. Five leaf-chambers were attached to one leaf of *Maranta coccinea*, var. *floribunda* at 4.30 p.m. on March 4, 1915. At 11.15 a.m. the next day, two were connected to two recording porometers, and the other three by means of a 4-way tube to a single bubbling aspirator for stop-watch readings (3), which were taken every fifteen minutes. The results are shown in Fig. 2, where the reciprocals of the distances between the two successive marks on the drum and the reciprocals of the stop-watch readings are plotted against time. The recorders of course gave continuous records, but as each curve would involve about 350 points, only one point for each five minutes period is given. It has been shown in some work which it is

hoped to publish shortly, that the stomata upon different parts of a leaf may be considered to behave similarly under similar conditions of illumination, &c., so that the curves in Fig. 2 are an indication of the relative accuracy of the recording apparatus and the ordinary porometer. The maxima in

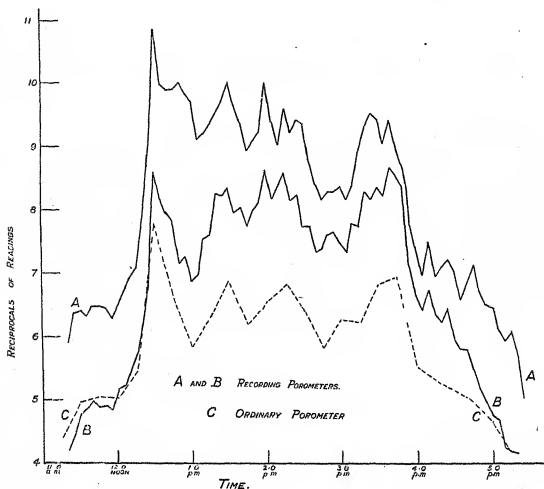


FIG. 2.

the curve of the latter are repeated in both recorder curves, and altogether here is a striking parallelism.

STOMATAL BEHAVIOUR DURING WILTING.

It has been stated by Darwin (4, 5) and Darwin and Pertz (6) that, in the case of plants investigated by them, on severing a leaf from the stem and allowing it to wilt, there occurs a temporary opening of the stomata prior to the closure following upon wilting. This preliminary opening has been demonstrated by the above authors by three different methods at different times—by the horn hygroscope, by temperature methods (in which, however, the assumption is made that the amount of transpiration from a leaf is an indication of the condition of its stomata), and also by the porometer method. Lloyd (7), using his alcohol method of stomatal

measurement, failed to find any indication of the temporary preliminary opening described by Darwin and Pertz.

The recording apparatus described above seemed to adapt itself well to a question of this kind, and consequently experiments were undertaken to investigate it.

The usual method adopted was to attach two leaf-chambers to different leaves of the plant to be investigated, and some hours later—generally the next morning—each was connected to a recording apparatus, and records were commenced. After an interval of an hour or more, one of the leaves was severed from the plant, the other being left attached to the plant to act as a control for temperature, humidity, and illumination changes. The experiment was continued, after severing one leaf, for a period determined by the result, and in many cases the second leaf was also severed later.

The plants used included *Maranta bicolor*, *M. coccinea*, var. *floribunda*, *Prunus Laurocerasus*, *Eucharis Mastersi*, *Eupatorium adenophorum*, *E. Raffilli*, *Pelargonium* (ivy-leaved), and *Phaseolus vulgaris*.

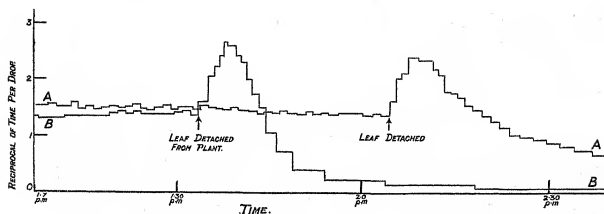


FIG. 3.

With the exception of *Eucharis Mastersi* all these plants showed the preliminary opening described by Darwin. Only one experiment was carried out with the *Eucharis*, and it is doubtful if the leaf was appreciably wilted at the end of the experiment, since the lamina is fairly fleshy and has a thick midrib which would hold much water, so that although no preliminary opening occurred in our experiment, further investigation might show that *Eucharis* is no exception.

Fig. 3 shows the result of one of these experiments upon *Phaseolus vulgaris*.

The time elapsing between severing the leaf from the plant and the opening movement of the stomata varies considerably with different plants and different conditions, and is apparently dependent upon the rate of wilting. In the case of a thin leaf which wilts quickly, the opening movement occurs very shortly after the leaf is cut from the plant, whilst in a thick leaf the opening may be long delayed. In the curves of stomatal

aperture shown in Fig. 3, the opening reached a maximum in both cases about five minutes after the severance from the plant, whilst in the case of the thick leaf of *Prunus Laurocerasus*, even when the temperature was 20°C. , tending to produce rapid wilting, nearly twenty minutes elapsed before the maximum opening was reached.

Similarly, with any particular plant, a higher temperature, tending to produce more rapid wilting, causes the maximum to be reached more quickly, and a roughly graded series may be obtained, as in the following experiments upon *Maranta bicolor*:

Experiment No.	Mean greenhouse temperature ($^{\circ}\text{C.}$).	Time elapsing before max. opening is reached.
100	16.3	35 minutes.
102 A	18.0	13 "
102 C	18.0	14 "
103	18.8	20 "
106	20.5	9 "

The extent to which the stomata open on severing the petiole is variable, depending probably upon the plant used, and possibly also upon the rate at which the leaf wilts. The greatest opening observed was with a leaf of *Phaseolus vulgaris*, in which the stomatal aperture, as deduced from the speed of the air-stream, being represented by the value 168 before cutting the leaf from the plant, increased to 475 within five minutes after cutting.

The results of these experiments are thus in close agreement with those obtained by Darwin and Pertz.

In his first reference to the phenomenon, Darwin suggested (4, p. 617) that the temporary stomatal opening is a direct result of wilting, and is due to the guard-cells retaining their water, and therefore remaining turgid longer than the other epidermal cells. Thus the pressure of the rest of the epidermis upon the guard-cells is reduced early in the process of wilting, with the result that the size of the stomatal pore increases.

The explanation offered by Darwin is no doubt the most probable one, but no experimental evidence was adduced in support of it, and alternatively there seem to be two other possible explanations which would fit the observed facts. The stomatal opening might be due, not to any effect of wilting, but to the shock sustained by the leaf as a result of severing it from the plant. In another connexion it has been demonstrated that the mere handling of some leaves may produce stomatal closure, but opening has never been observed to occur as the result of shock.

Another possibility was that the change in the porometer readings on detaching a leaf from the plant might be due to some cause other than stomatal opening.

In the unpublished work above referred to, it was observed that in

at least one plant, *Eucharis amazonica*, it was possible in a porometer experiment with a normal attached leaf, that some of the air drawn from the leaf entered it through the petiole and not through the stomata, since, when all the stomata outside the leaf-chamber were blocked, a current of air could still be drawn through the leaf unless the petiole was severed and blocked, e.g. by immersing in water.

It was thought possible therefore that when a leaf was detached from the plant and the petiole exposed, the air-stream might find the path through the petiole of less resistance than formerly. This would result in an increase in the speed of the air-current through the leaf, followed by a decrease when the stomata closed.

If this were the case, it is to be expected that the temporary 'opening' would reach a maximum almost immediately after detaching the leaf. The results show that some minutes elapsed in every case before the maximum was reached, which also confirms the observations of Darwin and Pertz.

This explanation of the phenomenon applies only to the results obtained by the porometer method, but Darwin's experiments with the horn hygroscope and temperature methods (4 and 5) showed clearly that an increase in transpiration occurred when the leaf was detached; this he adduced as further evidence for the view that the stomatal apertures had increased.

Dixon (8) has, however, suggested that the result of detaching a leaf is to reduce the tension in the water columns in the tracheae and thereby permit of more active evaporation from the mesophyll cells.

Experimental tests of these possible explanations were undertaken, using chiefly *Phaseolus vulgaris*, *Eupatorium adenophorum*, and *Maranta coccinea*, var. *floribunda*.

To prevent any flow of air through the petiole after severing it, the cut end was blocked by various means. Vaseline was found to be unsatisfactory owing to the difficulty of attaching it to the wet surface. Immersion in mercury, and coating the end with a wax mixture, stiff gelatine and stiff glue, were among the methods used to close the cut end of the petiole. The results showed that blocking the petiole by these means did not prevent the usual temporary opening, even though, in the case of the experiments with gelatine, the petiole was cut beneath the surface of liquid gelatine, and the cut end was therefore never exposed to the air. The phenomenon cannot therefore be attributed to the leakage of air through the petiole.

If, however, the petiole is cut below the surface of water, and kept supplied with water, the temporary opening does not occur. Instead there is either a slight tendency to closure, or else the stomata behave quite similarly to those of the control leaf still attached to the plant. Under these conditions the leaf does not visibly wilt in the course of several hours, or at most is only slightly less turgid than the leaves attached to the plant.

This result disposes of the possibility of shock being responsible for the

temporary opening, since severing the petiole beneath gelatine cannot cause more or less shock than severing it beneath water, and yet in the one case the opening occurs, and not in the other.

At the same time it is shown that when the leaf does not wilt the effect of severing it from the plant is practically nil.

From our experiments, therefore, it appears that when a leaf is detached from the plant and allowed to wilt, the stomata open for a short time before finally closing, and these results are quite in accordance with the observations of Darwin and Pertz.

In addition, some direct evidence has been obtained in support of the explanation of this phenomenon offered by Darwin, viz. that it is the direct result of wilting probably due to the guard-cells retaining their turgor longer than the other epidermal cells.

Neither stomatal closure due to shock nor the entrance of air into the leaf through the petiole can account for the increased porometer readings after detaching a leaf.

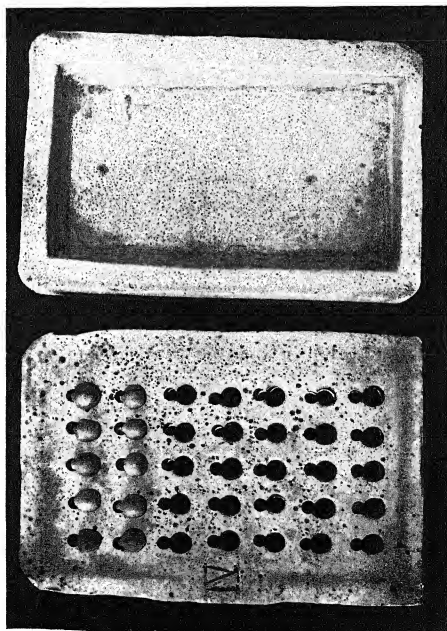
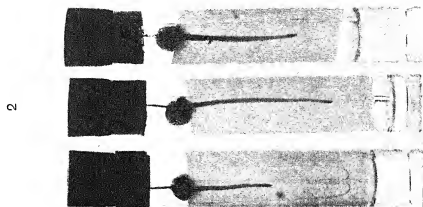
SUMMARY.

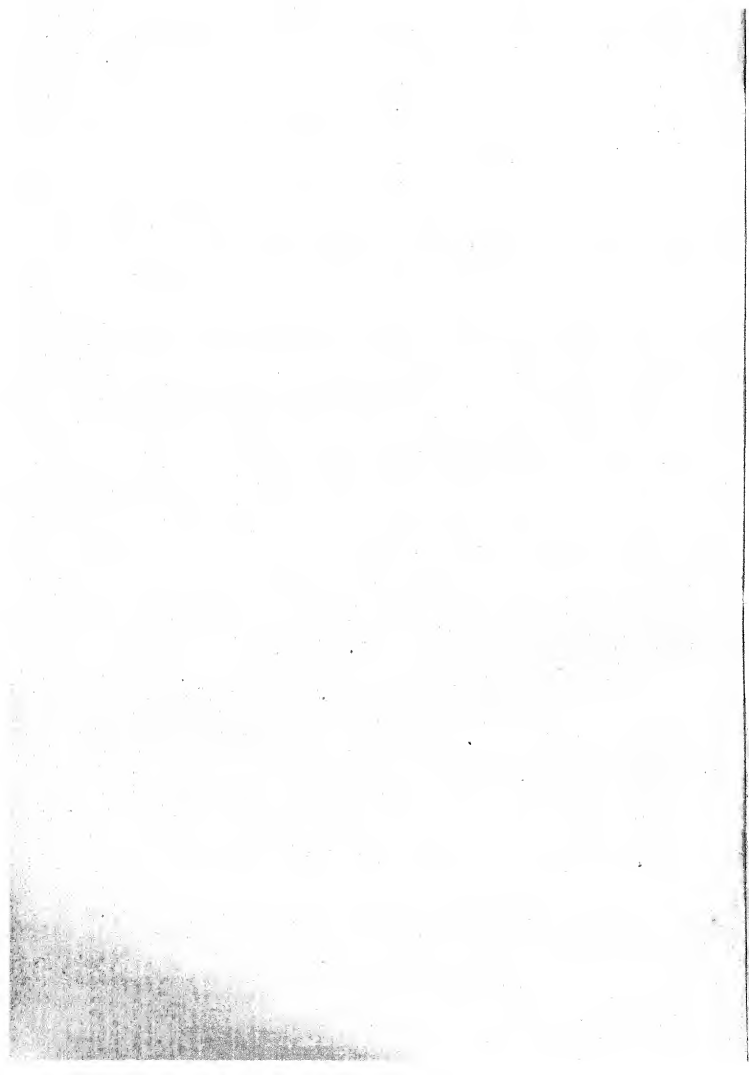
1. A description is given of a modification, making it self-recording, of the aspirator porometer described earlier.
2. Experiments on various plants confirm the observations of Darwin and Pertz—that on detaching a leaf from the plant and allowing it to wilt, the stomata open temporarily before finally closing.
3. Evidence is adduced in support of Darwin's suggestion that this phenomenon is due to wilting.

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On the Use of the Porometer in Stomatal Investigation.

BY

R. C. KNIGHT, B.Sc., D.I.C.

With seven Figures in the Text.

IN the course of some investigations upon transpiration it was found necessary to record the changes taking place, under varying conditions, in the size of the stomatal pores of the plants under observation.

The methods which have hitherto been employed for this purpose are various and widely divergent in principle. Some of the earlier observers assumed that the amount of transpiration from a plant was an index of the size of the stomatal apertures, and accordingly they expressed stomatal changes in terms of transpiration, as measured by experiments with the horn hygroscope (Darwin) (1) and cobalt chloride (Stahl) (2). Such methods must be rejected as untrustworthy in the absence of evidence in support of the assumption underlying them.

At the other extreme is the direct method used by Lloyd (for *Gossypium*) (3), in which the stomatal apertures are measured *in situ* in the living leaf by means of a microscope, a micrometer scale, and special illumination.

The two methods now most commonly used are :

- (1) Lloyd's earlier alcohol method (4) and
- (2) the indirect porometer method of Darwin and Pertz (5).

The relative values of these two methods have been discussed by the two latter authors (*loc. cit.*), the chief points considered being the advantage in Lloyd's method of taking observations over many different parts of the plant, and the advantages in the porometer of automatically averaging many stomata and of dealing with the same stomata throughout the experiment. In addition, the manipulation of the porometer is very simple, while, as will be readily admitted by any one who has attempted it, the accurate measurement of stomatal aperture under a microscope is very difficult and would seem to be liable to serious error.

In the light of these considerations, therefore, the porometer method was adopted for the work in question, but at the same time it was realized from an *a priori* analysis of the conditions of the experiment, that there was ample opportunity for errors to creep in unless suitable

precautions were taken to obviate them. Accordingly, some preliminary experiments were carried out with a view to determining these possible errors and the steps to be taken to avoid them. The results of these investigations are embodied in the present paper.

The majority of the experiments here quoted were carried out with the form of porometer described in a previous paper (6). The conclusions arrived at fall naturally into three main groups, and it is with these in view that the experiments have been classified, although many of them might be included in more than one group.

CHANGES IN STOMATA.

In dealing with such admittedly sensitive organs as stomata, it seemed possible that rough treatment might easily cause changes in the size of the pore, and some experiments were therefore carried out to determine whether the conditions of the porometer experiment affected the stomata. The main source of possible error appeared to be changes in stomatal size resulting from:

- (1) the mechanical strain on the leaf due to the reduced pressure ;
- (2) the passage of the air-stream through the leaf ; and
- (3) the effect of shock from the manipulation necessary for fixing the leaf-chamber.

(1) One aspect of the question of mechanical strain has already been discussed in a previous paper, where it was pointed out that even a small pressure difference will cause a curvature of the portion of the leaf under observation, and this effect cannot therefore be neglected. Although there is no direct evidence on the question, it is possible that the stomatal pores in general will be affected by a change of curvature of the leaf. The acceleration (or retardation) of the air-current through the leaf, caused by this stomatal change, will obviously be incorporated in the porometer readings, and will constitute a regularly occurring error. If, however, the pressure difference employed to produce the air-current is maintained constant throughout an experiment, the leaf-curvature, and consequently the error resulting, will also be constant. Since this error will have a different value for every plant, perhaps for every leaf, and for each different pressure, it seems hopeless to attempt to determine it.

As Darwin pointed out, the porometer gives comparative measurements of the direction and relative amount of stomatal changes, but not the actual dimensions of the pore ; this being so, a constant error, if small, will not detract from the comparative value of the readings, so that if the pressure differences used are as small as is convenient the curvature of the leaf is a factor which may be neglected.¹

¹ By supporting the leaf by means of a grid placed between it and the chamber the curvature might, no doubt, be avoided.

(2) Errors arising from the effects of the passage of the air-stream through the leaf are, from their nature, unavoidable, but a few experiments have been carried out in reference to them.

One effect of the air-stream is to replace the moist air in the inter-cellular spaces by drier air from without, which will result in an increase of transpiration from the leaf. If this increase is sufficiently large, it might conceivably result in wilting and stomatal closure, but if the air-stream is passing for only a short period—the usual procedure—the effects must be practically negligible.

An attempt was made to determine experimentally any after effect resulting from continued passage of the air-stream through a leaf. Two or more leaf-chambers were attached to different leaves of the same plant, and porometer readings taken at intervals. The leaves were considered as mutual controls, the justification for which will appear at a later stage in this paper. Except when readings were actually being taken the leaf-chambers were in direct connexion with the outer air, so that there was no undue pressure upon the leaves and no artificial air movement within them. Having by this means determined the direction and slope of the curves of stomatal movement of each leaf during a period of two hours or so, air was drawn through one leaf at constant pressure for an hour or more, the periodic readings being continued for all the leaves. In some experiments the air-stream was then transferred to other leaves, the first leaf now acting as a control. By this means it was expected that any effect the air-current might have upon the stomata would be reflected in the readings during, and after, the period in which the stomata were subjected to a continuous stream.

The plants used were *Ficus elastica*, *Eucharis Mastersi*, and *Begonia* ('President Carnot'), and the results were not the same for the three. *Ficus* and *Eucharis* were quite unaffected by the air-stream, whereas in the case of *Begonia* the stomata showed a distinct and sometimes considerable closure as a result of drawing air through, and when the stream was stopped there was a tendency for the curve to return to its former slope. Figs. 1 and 2 show curves obtained in experiments with *Begonia* and *Eucharis* respectively.¹

Thus, although the conditions of the experiment were somewhat severe as compared with the ordinary porometer experiment, it is obvious that stomata may be affected by the air-stream used in the latter. The resulting error will be reduced to a minimum by ensuring that between two readings the air-current is stopped, and that, in addition, the time occupied in taking a reading is reduced to a minimum.

¹ These and all other curves in this paper were obtained by plotting the reciprocals of the time between successive bubbles, not the square roots of the reciprocals, which was the method mainly adopted by Darwin and Pertz.

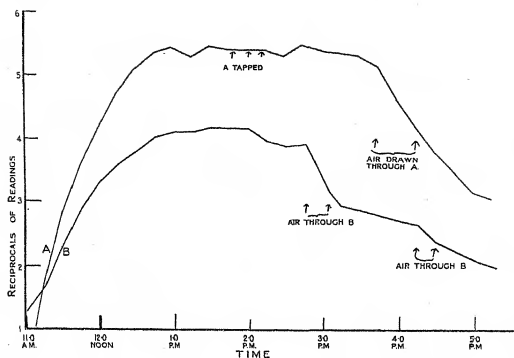


FIG. 1. Effect upon the stomata of the continued passage of air. *Begonia* ('President Carnot').

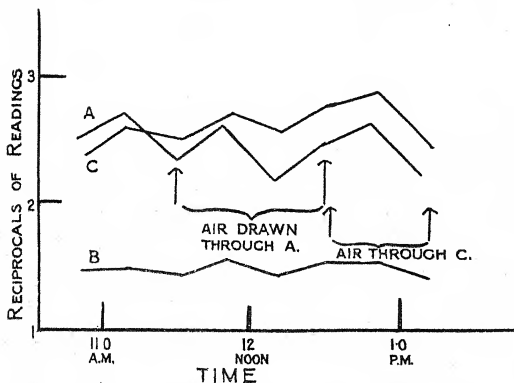


FIG. 2. Effect upon the stomata of continued passage of air. *Eucharis Mastersi*.

In addition to the above-mentioned stimulation, which partakes of the nature of a cumulative effect, there is the possibility that a change in the stomata may be produced immediately the air-stream begins to pass through a leaf, an effect which is unaltered by the continued appli-

cation of the stimulus. Such an effect may be the result of forcing apart resilient guard-cell walls which return to their original conformation immediately the rush of air ceases. By the porometer method it is obviously impossible to detect such an error, and equally impossible to avoid it, but the existence of any large error of its kind seems unlikely.

(3) In view of the fact that a porometer experiment involves the attachment of the leaf-chamber to the leaf by means of some adhesive, a somewhat violent treatment, some experiments were carried out to obtain some indication of the response, if any, of the stomata to shock.

In some experiments, in another connexion, it was observed that vaselining the upper surface of a leaf of *Acalypha Wilkesiana* var. *marginata* with a brush caused rapid closure of the stomata, which are confined to the lower surface. This was attributed to shock, but the experiments were not followed up.

In later experiments it was frequently noticed that shortly after fixing the chamber to the leaf, the size of the stomatal apertures, from porometer readings, was much less than, in the light of later readings from the same portion of leaf, seemed consistent with the general conditions of atmosphere and illumination. It was thought that, possibly, this was due to the fact that the manipulation necessary for the attachment of the chamber to the leaf caused the stomata to close. Confirmation of this was sought in a series of experiments, all of which gave the same results; one of which is given in detail below.

Expt. 56. 15. xii. '14, and 16. xii. '14.

The plant used was *Eucharis Mastersi*, and at 2.0 p.m. on 15. xii. '14 two similar leaf-chambers, B and D, were fixed to different parts of the same leaf. Readings were taken at intervals from 2.30 p.m. onwards, and showed that the stomata opened rapidly until about 3.30 p.m., when they began to close, doubtless owing to the failing light (see Fig. 3). The readings were continued the next morning and throughout the day. At 11.35 a.m. another chamber, C, was attached to the leaf and readings taken from it as from the others. The most important point revealed in the resulting curves is that the stomata at C were at 12.10 p.m. almost closed, but they rapidly opened to a maximum at 1.30 p.m. at the same time as B and D, finally closing in unison with them.

It may be assumed that the stomata at C behaved similarly to those at B and D on the morning of 16. xii. '14, and that at 11.35 a.m. they were fairly widely open, but that they closed in response to the shock sustained in fixing the chamber C, but recovered by 1.30 p.m. The same conclusion applies to the closed state of the stomata at B and D at 2.30 p.m. on 15. xii. '14.

¹ N.B.—The reason for lack of absolute coincidence, even under practically identical conditions, between B and D is discussed in a later section of this paper.

The results of this series of experiments show that the treatment to which a leaf is subjected in affixing a chamber produces a definite stimulation of the stomata, causing them to close to a large extent. Recovery from the shock is fairly rapid, taking place in two hours or so, and is apparently complete, since it is found that a chamber can be left attached to a leaf for days, and readings may be taken daily, whilst the stomata still respond to light and darkness, and the leaf is not obviously injured.

A second method was also adopted in the attempt to discover the effect upon stomata of shock. Two or more chambers were attached to

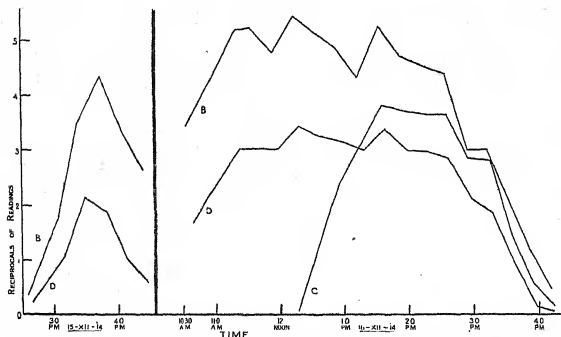


FIG. 3. Effect upon the stomata of fixing a porometer chamber. *Eucharis Masteri*.

different leaves of a plant, and when the curves of stomatal aperture had attained a regular form, one of the leaves under observation was tapped lightly several times with a pencil, or was shaken, and the effect upon the stomata was noted in the succeeding readings. It is naturally somewhat difficult to standardize tapping or shaking, and it was therefore impossible to treat each leaf similarly. It is possible that this accounts for the variety of results obtained.

Begonia showed no effect at all, the curves of stomatal aperture having similar forms whether the leaf was tapped or not. *Ficus elastica* showed no change in two cases, but in a third there was a tendency for the stomata to close as a result of tapping. *Eucharis Mastersi* fairly consistently closed its stomata, temporarily, in response to tapping. With all three plants several experiments showed slight effects which might be due to merely experimental error,

Therefore to avoid errors from this source it is advisable to touch or shake the leaf as little as possible after affixing the leaf-chamber. It was with this end in view that a three-way stopcock was inserted between the leaf-chamber and the aspirator bottle in the apparatus used for the porometer readings (6); for by this means the chamber can be connected with the outer air with the minimum risk of shaking.

It is obvious that Lloyd's alcohol method of measuring stomata is not applicable to plants with sensitive stomata, since the manipulation required by his technique would be sufficient to cause change of aperture.

EFFECT OF INTERCELLULAR SPACES.

The air drawn through the leaf in a porometer experiment must enter the leaf through stomata situated outside the leaf-chamber, must pass through a length of intercellular spaces, and finally leave the leaf through the stomata covered by the chamber. The rate at which this air moves is determined by the pressure difference, and by the resistance encountered, part of which is due to the stomata and part to the intercellular spaces.

Any change in the size of the stomatal pores will be reflected in the resistance they offer to the air-stream, and therefore in the speed of the air, if the size of the intercellular spaces remains constant. In interpreting the rate of air-flow as an indication of stomatal aperture, this constancy is necessarily assumed, but it would seem probable that when a leaf wilts, for instance, the intercellular spaces will be appreciably smaller than when the tissue is turgid. But in a normal daily cycle of changes, the variation is likely to be small, and the influence the spaces have upon the readings will depend upon the relative resistance to air-flow of stomata and spaces respectively. If the resistance is mainly due to the stomata, changes in the intercellular spaces will have but little effect upon the readings, but if a considerable proportion of the resistance is due to the spaces, then their changes will be reflected in the readings, and, in addition, changes in the size of the stomata will be appreciably masked by the part played by the spaces, and the sensitiveness of the method will be impaired thereby.

The following series of experiments constitutes an attempt to estimate the relative importance of the parts played by the stomata and intercellular spaces in resisting the air-flow. From the nature of the problem, an accurate estimate of the relationship is almost impossible, especially as it is probably different for every plant and is continually changing, so that no quantitative generalization has been made.

Four methods have been employed :

1. Two chambers were fixed to the same leaf some distance apart, and periodical readings taken from each, preferably till a fairly constant value was reached in both. Vaseline was then smeared on that portion of the

leaf immediately adjacent to one of the chambers, so as to block the stomata over an area in the form of a ring around the chamber. This treatment increases the length of intercellular spaces to be traversed by the air-stream, and the difference between readings taken before and after vaselining will be due to the added resistance of the extra intercellular spaces, provided that the control chamber gives readings similar to those taken previously. By vaselining a portion of the leaf-surface a number of stomata are blocked, but as long as the unvaselined area is considerably greater than the area under the chamber the rate of the air-stream will not be limited by the unvaselined portion.

Expt. 8. 2. xi. '13.

Two chambers, A and B, fixed one on each side of the midrib of leaf of *Eucharis Mastersi*.

Area of leaf under chambers: A, 1.4 sq. cm.; B, 1.3 sq. cm.

Area of leaf unvaselined outside the chambers:

on A side of midrib, 106 sq. cm.

„ B „ „ „ 123 „

Width of ring of vaseline, approx. 0.8 cm.

Thus the increase of length of intercellular spaces to be traversed was 0.8 cm., and the unvaselined area was 75 to 95 times the area under the chamber.

TABLE I.

The readings are given in seconds and represent the time required to draw a given volume of air through the leaf. The vaseline was applied at 12.30 p.m. B was the control.

A.		B.	
Time.	Reading.	Time.	Reading.
12.15 p.m.	12.8	12.17 p.m.	11.7
12.25	12.9	12.27	11.7
12.30 p.m.	[Vaselined]	—	—
12.37	23.1	12.40	13.1
12.47	20.1	12.50	11.6
12.57	17.1	1.0	10.7
1.25	15.7	1.27	9.8
2.8	16.1	2.10	10.5

The immediate effect of vaselining was to reduce the rate of air-flow in both cases, which was to be expected in view of the fact already established that handling causes stomatal closure in *Eucharis Mastersi*. The decrease in rate was however much greater for A than for B, and by 2.10 p.m., when both had apparently recovered and the stomata were closing, presumably in response to some natural stimulus, the air-stream in A was still much slower than in B. By comparing in the case of A the two lowest readings (12.8 and 15.7) we find that the rate has decreased by about 20 per cent. as a result of increasing the length of intercellular space traversed by only 0.8 cm., at

the same time the control reading has changed from 11.7 to 9.8, representing an increase of 16 per cent. in the rate; the effect of vaselining is clearly much greater than the readings of A indicate.

A modification of this method has also been employed with similar results. In this case the whole leaf outside the chamber was vaselined, except a long rectangular area, the shorter side of which was immediately adjacent to the leaf-chamber. Readings having been taken, the length of intercellular spaces to be traversed was increased by vaselining a portion of the rectangle nearest the chamber. In this case an increase of 2.0 cm. produced a decrease in rate of 35 per cent., the control remaining practically constant throughout. The plant used was *Eucharis amasonica*, which, as well as the other species of *Eucharis* used in the experiment previously described, has stomata only on the under surface of the leaves, with occasional ones on the petiole. The case of hypostomatous plants of this type is somewhat different from that of plants with stomata upon both leaf-surfaces, such as species of *Helianthus*, *Saxifraga*, and *Richardia* which have been used in later experiments. In the latter types the air-stream is at liberty to enter the leaf from one side and pass straight through into the chamber on the other side, reducing the length of intercellular spaces traversed to the thickness of the leaf. A vaselining experiment, as described above, upon an amphistomatous leaf would require the whole of the

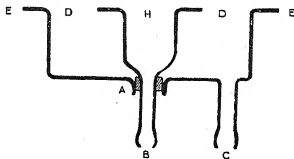


FIG. 4. Diagram showing the form of double chamber used.

stomata upon one surface to be blocked before measurements could be made. The question of amphistomatous leaves will be further discussed later.

2. A more convenient and efficient method of carrying out what is practically the same experiment as that described above has since been used. It depends on the use of a special type of double chamber of the form shown in Fig. 4. This consists of one chamber within another, so arranged that both can be fixed to the same leaf, each being provided with an independent outlet. Those used in the following experiments have been generally constructed in two parts and joined by means of rubber tubing or sealing-wax (at A in Fig.). The double chamber is fixed to a leaf and readings are taken from the inner chamber in the usual fashion. The air enters the outer chamber at C, passes into the leaf at D, and is drawn into the inner chamber at H. This constitutes an ordinary porometer reading from a single chamber. The outlet C of the outer chamber is now closed, and air must now enter the leaf at E—beyond the outer chamber, and to reach the inner chamber must traverse a longer path within the leaf.

Readings are again taken, and the differences between these and the previous ones are an indication of the added resistance due to the extra length of intercellular spaces through which the air has now to pass.

This method is easier of manipulation than the vaselining one, in that the extra length of tissue can be introduced at will and without any shock to the plant, an important consideration in view of the effect of shock upon the stomata already described.

In constructing the double chamber it is advisable to ensure that the area of leaf between the outer and inner chambers (i. e. DD in Fig.) is somewhat greater than the area covered by the inner chamber (H), so that, when the outer chamber is open, the conditions may closely approximate to the ordinary single-chamber experiment. If DD is greater than H, there will be less tendency for air to enter the leaf at EE when C is open.

The usual method of procedure was to take three readings in succession with the outer chamber open, the mean of these representing the stomatal resistance plus the resistance of a short length of intercellular spaces. The outer chamber was then closed and readings taken continuously till they became constant. Finally, the outer chamber was again opened and three more readings taken, in order to allow for stomatal changes during the experiment, the mean of the first and last three being considered to be the correct 'open' reading.

It was found that readings taken immediately after closing the outer chamber were not always the same as those taken a few minutes later, and this was attributed to the fact that at the moment of closing the outer chamber the air in it was at atmospheric pressure, but before any equilibrium could be set up the pressure in the outer chamber must approach that in the inner one.

The difference between the 'open' and 'closed' readings was expressed as a percentage of the 'open' reading, this latter being regarded as the closer approximation to the indication of stomatal resistance. For each leaf investigated, several of these composite readings were taken at different times of the day, corresponding to different dimensions of stomatal aperture, and the series of percentages thus obtained were plotted as ordinates with the reciprocals of the 'open' readings as abscissae, i. e., roughly, the effect of the added length of intercellular spaces was plotted against stomatal aperture.

It must be noted here that this method of plotting is entirely arbitrary, and the figures obtained only refer to one particular set of dimensions of the chambers used, but by expressing the results in this manner the conclusions drawn from them are easily demonstrated.

Experiments with double chambers have been carried out with a variety of plants, including *Hedera helix*, *Eucharis amasonica*, *E. Mastersi*,

Ficus elastica, *Phaseolus vulgaris*, *Helianthus annuus*, *Begonia* ("Gloire de Lorraine"), *Begonia* ("President Carnot"), *Aucuba japonica*, *Richardia aethiopica*, *Saxifraga cordifolia*, *Morus nigra*, *Eupatorium Raffilli*, *Dracaena Godseffiana*, and *Piper dilatatum*.

These are hypostomatous plants with the exception of *Helianthus*, *Richardia*, *Saxifraga*, and *Dracaena*, which are amphistomatous. The following experiment shows a typical result obtained with a hypostomatous leaf.

Expt. 38. 26. xi. '14.

A double chamber attached to leaf of *Begonia* ("President Carnot"). The dimensions of the chamber were as follows:

External diameter of inner chamber, 1.0 cm.

External " " outer " 3.5 "

so that the extra length of path traversed by the air when the outer chamber was closed was 1.25 cm. approx. Readings were taken during both the morning opening and evening closing of the stomata. The curve obtained is shown in Fig. 5.

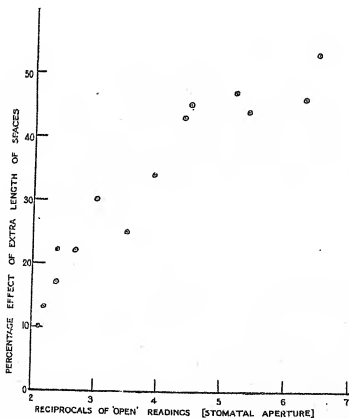


FIG. 5. Effect of the intercellular spaces of the leaf upon porometer readings.

The points do not lie upon a smooth line, but this is not surprising since slight changes in the size of the intercellular spaces are probably of frequent occurrence. The chief point for consideration is that, with increasing size of the stomatal aperture, the relative part played by the

intercellular spaces also increases. This has been the case in all the hypostomatous leaves investigated, and is in complete accord with expectation. As the stomata open, the resistance offered by them to the air-flow decreases, and that offered by the intercellular spaces remains approximately constant, so that, relative to the total resistance, that of the intercellular spaces increases as the stomata open.

Another phase of the same phenomenon is evident in the differences in different plants. A plant whose stomata are large or are capable of wide opening, such as species of *Eucharis*, shows relatively a much greater intercellular space resistance than a plant with fewer or smaller stomata, as *Eupatorium Raffilli*. With the double chamber of dimensions stated above, 59 per cent. was the highest value obtained for the relative resistance of the spaces in *Eucharis amazonica*, whilst in *Eupatorium Raffilli* the value never rose above 18 per cent.

The case of the amphistomatous leaf is somewhat different from that of the hypostomatous one. The double-chamber experiment with the former has not the same significance as with the latter, unless the stomata upon the surface opposite to that on which the chamber is fixed are blocked.

Experiments of this type have been performed with the amphistomatous leaves mentioned in the above list, and all of them gave results comparable with those obtained with hypostomatous leaves.

In an ordinary porometer experiment with an amphistomatous leaf, there is available for the air-stream a path directly through the leaf from one surface to the other, and it is probable that this direct path offers less resistance than the longer one which the air necessarily traverses in a hypostomatous leaf. It is therefore likely that the air will take the path of least resistance and pass directly through an amphistomatous leaf. A test of this can be carried out by a double-chamber experiment without previously blocking the stomata of one surface. If the resistance of the direct path is relatively small, then the greater part of the air will pass along it, and closing the outer chamber will have little or no effect upon the readings; but if the resistance of the direct path is relatively large, some portion of the air-current will be diverted by closing the outer chamber, and a decrease in speed will result.

In the case of *Richardia*, when neither surface was vaselined, the effect of closing the outer chamber was never more than 3 per cent.—probably within the limits of experimental error—but when the stomata on one surface were blocked, the effect rose as high as 29 per cent. *Dracaena*, on the other hand, showed an effect up to 12 per cent., whether one surface was vaselined or not, the double chamber used having the same dimensions as that previously described.

3. Owing to its simplicity of manipulation, the method just described

has been used for a variety of plants, whilst other less simple methods have been used in one or two experiments on suitable plants. Of these the following method provides clear demonstration of the part which intercellular spaces may play in retarding the air-stream.

Several chambers (four and five have been used) are fixed in a straight line on one leaf, preferably of uniform nature. Small chambers or a large leaf must be employed—*Eucharis Mastersi* and *E. amazonica* have been found to answer well with chambers of 5 mm. internal diameter. The leaf is now smeared with vaseline over the whole stoma-bearing surface, except for a small area, 1 sq. cm. or so, just beyond the end chamber of the series and in line with them. This area serves for entrance to the leaf of the air-stream, which is drawn out through the chamber at the other end of the line by a constant-pressure aspirator in the usual manner. Each of the intermediate chambers is connected to a glass tube dipping into water and serving as a manometer.

A stream of air entering the leaf at F and leaving it at A (see Expt. 52, p. 70) will encounter the resistance of the stomatal pores at those points, and also that of the tissue between them. If this latter resistance is not appreciable, the passage of the air from F to A within the leaf will be easy, and the air pressures in the intercellular spaces at F and A will not be widely different. If, on the contrary, the resistance offered by the tissues is considerable, there will be a pressure-gradient in the leaf along the line FA, which will be indicated by the manometers B, C, D, E. In practice it is found that the movements of the water-columns in the manometers are slow, owing to the pressure having to be transmitted through the stomata, so that air is drawn through the leaf continuously for some hours and the manometers read at intervals. A rough estimate of stomatal changes is deduced from the rate of the air-stream.

In all experiments in which extensive blocking of the stomata was resorted to—generally with species of *Eucharis*—it has been found expedient to cut the leaf from the plant and immerse the cut end of the petiole in water, on account of the tendency of air to pass through an attached petiole from other parts of the plant, when air is drawn from the leaf into the leaf-chamber. This can be demonstrated by fixing a chamber to a leaf and vaselining the whole of the rest of the surface, when it will still be found possible to draw air into the chamber. If the petiole is now severed and the end immersed in water, the air-stream stops. On cutting the petiole again above the water, air can be again drawn through, showing that the previous cessation of the stream was not due to stomatal closure.

There is little possibility of the experiment being affected by severing the leaf, as is shown by the fact that three days after a leaf was detached and its petiole immersed, the stomata were still responding to the daily changes of illumination.

Expt. 52. 10. xii. '14.

The plant used was *Eucharis Mastersi*. Five chambers (A, B, C, D, E) were fixed to a leaf and the leaf vaselined as described above. The chambers were about 2.2 cm. apart. Pressure difference in the aspirator was 18.5 cm. Manometers were read every thirty minutes; the maximum heights reached, excluding capillary effects, were:

B.	C.	D.	E.	F.
10.0 cm.	8.7 cm.	7.3 cm.	6.3 cm.	(unvaselined area)

Rough approximations from these figures give the pressure inside the leaf at A as 11.3 cm. below an atmosphere, and at F 5.5 cm. below, i.e. the difference of pressure on the two sides of the barrier of the stomata was 7.2 cm. (18.5-11.3) at A, and 5.5 cm. at F, the lack of agreement probably being due to the greater area of leaf exposed at F.

Thus, whilst in passing from the leaf through the stomata into the chamber the pressure change is 7 cm., in passing through a centimetre of leaf-tissue the pressure change is at least 0.5 cm., showing that the intercellular spaces may offer a relatively large resistance to the air-current.

4. An extension of the pressure-gradient experiment has also been used to supplement the methods already described. A series of chambers is attached to a leaf, and the remaining leaf-surface is smeared with vaseline. The end chamber of the series is connected with the aspirator, and the others are provided with stopcocks so that air may be allowed to enter the leaf through any particular chamber at will. Whichever path the air-stream is made to traverse, it encounters two sets of stomata, one at the entrance and one at the exit, but the length of intercellular spaces to be traversed may be varied at will by means of the stopcocks, and the difference in speed caused by different lengths can be measured in the usual manner.

It must be noted that for the correct working of this experiment the areas exposed under the leaf-chambers must be equal, and to ensure this is far from easy in practice. If, however, the areas are approximately equal, and if the areas beneath chambers farther from the exit chamber are not less than those beneath chambers nearer to it, the experiment will have a qualitative significance.

Expt. 53. 11. xii. '14.

Five chambers, A, B, C, D, E, fixed to a leaf of *Eucharis Mastersi*, and the remainder of the leaf-surface vaselined.

The areas of leaf exposed under the chambers were in the proportion:

A.	B.	C.	D.	E.
10	25	25	20	10

and the distances between the chambers are indicated below:

A. 2.3 cm.	B. 2.3 cm.	C. 2.3 cm.	D. 1.9 cms.	E.
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E was connected to the aspirator with a pressure difference of 15 cm.¹ Readings were taken with each of the chambers open in turn, but those from A were discarded, as indicated above. Only one set of readings is given here, which are typical of those throughout the experiment.

TABLE II.

<i>Path of air-current.</i>	<i>Length within leaf.</i>	<i>Time between bubbles.</i>
D to E	1.9 cm.	12.9 sec.
C to E	4.2 „	15.6 „
B to E	6.4 „	18.7 „

A variety of methods having been used to demonstrate the resistance of the intercellular spaces, some consideration of the effect of this resistance upon porometer readings is necessary.

In fixing a porometer chamber to a leaf a large number of stomata are of necessity blocked by the adhesive, and thus in a hypostomatous leaf the stomata by which the air enters are some distance from those by which it passes from the leaf into the chamber, and an extra resistance is therefore encountered. In an amphistomatous leaf the blocking of the stomata is of less account, as the air may pass from one surface to another, although, as has been shown (p. 68, *Dracaena*), this direct path is not always traversed.

For accurate work with hypostomatous leaves it is thus important to reduce the length of path within the leaf by blocking as few stomata as possible.

In their original paper on the porometer (loc. cit.), Darwin and Pertz described the leaf-chamber used by them as having a flange at the mouth to facilitate its attachment to the leaf. Balls (7), in his work on the Stomato-graph, advocates a form of chamber which also involves blocking the stomata over a very considerable area. These forms may be suitable for amphistomatous leaves, but if used for others must introduce much intercellular space resistance, and tend to mask stomatal changes, if not actually to introduce errors.

The form of chamber which has been generally found efficient is a piece of glass tubing of the required bore, tapering at one end to take the rubber connexion, and with the other end cut off square and ground flat, leaving to be attached to the leaf a surface equal in width to the thickness of the walls of the tubing, i.e. 1 to 2 mm.

Darwin and Pertz, after experimenting with many adhesives, finally decided in favour of glue, whilst Balls has recommended paraffin. In the present work glue has been found quite satisfactory, but the consistency needs to be carefully adjusted, depending upon the plant used and the

¹ In practice, readings were also taken using A, B, C, and D respectively as the exit chamber, and only after the experiment, when measurements of areas had been made, was it possible to determine which set of results was significant.

temperature. Only occasionally was a leaf found to be injured as a result of fixing the chamber; after recovery from the first shock, leaves generally remained quite healthy with the chamber attached.

BEHAVIOUR OF STOMATA OF DIFFERENT REGIONS.

There is another question with reference to the porometer method which has been mentioned by Darwin and Pertz and also by Balls, viz. the relative condition of stomata on different parts of a plant. Porometer readings indicate stomatal changes only in the immediate vicinity of the leaf-chamber, and Darwin and Pertz quoted a set of readings from a plant of *Prunus laurocerasus*, which showed marked differences in the size of the stomatal pores in leaves of different age. Balls proposed to get over the

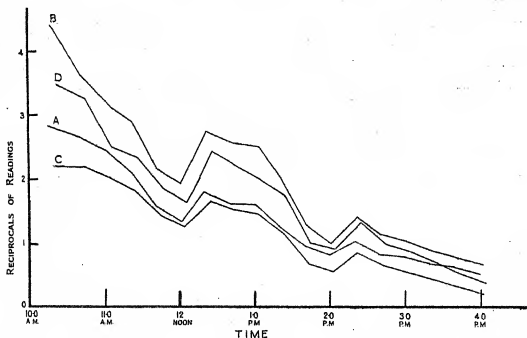


FIG. 6. Behaviour of stomata of different portions of a leaf under similar conditions. *Ficus elastica*.

difficulty by fixing several chambers to different parts of the plant under observation, and by connecting them all to one reading apparatus, a mean is automatically obtained. In this case, however, each chamber should include approximately the same number of stomata, otherwise changes in the stomata beneath the chamber including the greatest number are liable to take an undue share in the total result.

Experiments have been carried out on the behaviour of stomata on different parts of a leaf, and on different leaves of the same plant.

1. A series of similar chambers were fixed to different parts of a leaf, and periodical readings taken from each. To determine the quantitative relations of stomatal aperture in the different regions, the reading per unit area for each chamber was calculated from the area of leaf exposed and the average reading over the whole period.

Expt. 54. II. xii. '14 and 12. xii. '14.

Four chambers, A, B, C, D, were attached to one leaf of *Ficus elastica* at 11.0 a.m., II. xii. '14, A, C and D being on one side of the midrib at a distance from the apex of about one-quarter, one-half, and three-quarters, respectively of the length of the leaf. B was on the other side of the midrib, about one-third of the leaf-length from the apex.

Readings were taken at intervals from 12.40 p.m. till 4.30 p.m., December 11, and from 10.15 a.m. till 4.0 p.m. on December 12. The curves in Fig. 6 are the results of the latter series, the first series, which is not given, showing the closure due to shock and the subsequent recovery, which has already been discussed and illustrated. In the curves here shown there is not absolute coincidence, probably partly due to the different areas included under the respective chambers, but there is a very striking parallelism in all four curves, a change of direction in one being accompanied by similar changes in the others. The frequent irregularities were probably due to the varying illumination.

The areas and mean readings were :

	A.	B.	C.	D.
Area	1.0 sq. cm.	1.2 sq. cm.	0.8 sq. cm.	1.1 sq. cm.
Mean reading . . .	0.142	0.171	0.117	0.167
Reading per unit area	0.14	0.14	0.15	0.15

These show good agreement, and indicate that if the numerical distribution of stomata over the leaf-surface is regular, the stomatal pores in different regions of the leaf are open to the same extent under similar conditions.

The distribution of stomata will be discussed later.

Experiments upon *Eucharis Mastersi* and *Eupatorium Raffilli* gave similar results, and although exceptional readings are often found, it may be assumed that as a general rule in these plants the readings obtained with a porometer from one chamber on a leaf are indicative of the condition of stomata over the whole leaf.

2. The type of experiment just described was repeated, with chambers on different leaves instead of on one leaf, with comparable though not exactly similar results.

Expt. 70. I. ii. '15.

Three similar chambers were fixed to different leaves of *Eucharis Mastersi* at 1.0 p.m. on 31. i. '15.

A, to an old leaf, brown at the edges.

B, to a mature healthy leaf.

C, to a young leaf.

Fig. 7 shows the curves obtained. These do not show the same parallelism as those in Expt. 54 previously described, but there is the same

tendency for changes in one curve to be accompanied by similar changes in the others.

	A.	B.	C.
Area . . .	1.0 sq. cm.	1.0 sq. cm.	1.0 sq. cm.
Mean reading	0.128	0.317	0.193

It is clear that the stomata of the mature healthy leaf were more widely open than those of the very young or very old leaves, whilst from the curves it may be seen that the stomata of the mature leaf were capable of closing to an aperture as small as those of the other two.

Similar results were obtained with *Ficus elastica* and *Eupatorium Raffili*.

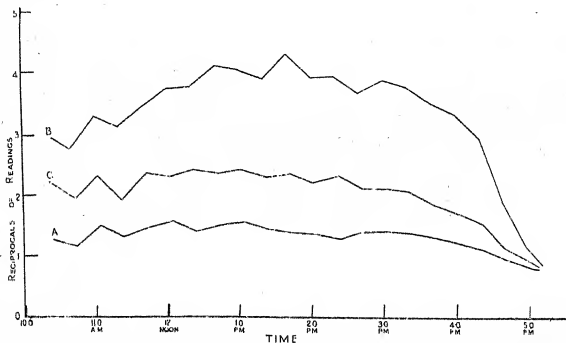


FIG. 7. Behaviour of stomata on different leaves under similar conditions. *Eucharis Mastersi*.

The generalizations as to the relative size of stomatal pores on different parts of a plant are made on the assumption that the stomata are evenly distributed over all leaves. To test this, several countings were made of the number of stomata per unit area of epidermis on leaves of *Eucharis Mastersi* and *Ficus elastica*.

From the results it appears that the region nearest the edges of the leaf, particularly near the apex, is more thickly covered with stomata than the more central portions, in some cases the ratio being as large as 1.4 : 1.0; but at quite a short distance from the edge the numbers are almost the same as those near the midrib.

In the case of leaves of different ages there is a distinct gradation—the old leaves having most and young leaves fewest stomata per unit area, the largest ratio observed being 1.3 : 1.0.

Thus in the porometer experiments upon one leaf only, the chambers were never near enough to the margin to be affected by the irregular

distribution of stomata, and in the experiments upon leaves of different ages, when the distribution is allowed for, there is still the same relationship between the mean readings.

Therefore, whilst readings from one chamber are adequate indication of stomatal behaviour in a single leaf, yet when the whole plant is considered it is advisable to fix chambers to more than one leaf, as suggested by Balls.

SUMMARY OF RESULTS.

The conditions of the porometer experiment involve the possibility of a number of errors which by means of suitable precautions can to a large extent be eliminated.

1. Temporary deformation of the leaf is liable to occur owing to the pressure difference employed to draw air through. Such deformation may cause undesirable changes in the stomatal pores. Any such effect is reduced to a minimum by using small pressure differences, and if a constant pressure is maintained the effect will be constant.

2. Some stomata show a tendency to close when air is drawn continuously through them. To avoid this, the air-current should be stopped when readings are not being taken, by placing the leaf-chamber in direct connexion with the outer air.

3. The stomata of some leaves are sensitive to shock, the handling involved in fixing chambers to the leaf causing the stomata to close almost completely, but recovery is fairly rapid, and two hours has been found sufficient. The mere tapping or shaking of some leaves may induce a closure of the stomata. It is therefore advisable that after the leaf-chamber is fixed, readings be not taken for two hours, and that the leaf be disturbed as little as possible.

4. The resistance offered by the intercellular spaces to the passage of air through a leaf is considerable, and may have a marked effect upon the porometer readings. In leaves other than amphistomatous ones, it is therefore advisable to reduce as far as possible the length of tissue to be traversed by the air-stream, by using chambers of suitable construction.

5. In the plants investigated, stomata on different parts of the same leaf behave similarly under approximately similar conditions, and are open to about the same extent at the same time. Thus readings from one chamber on a leaf are sufficient indication of the stomatal behaviour of that leaf.

6. Stomata on different leaves in general behave similarly, but the agreement is not so close as between stomata on the same leaf. Stomata of a mature healthy leaf may open more widely than those of either a very young or very old leaf. Thus, when using a plant with several leaves, in order to obtain a comprehensive measure of the behaviour of its stomata, chambers

should be attached to more than one leaf, but for reasons already stated the chambers should be about the same size.

It is with great pleasure that I record my indebtedness to Professor V. H. Blackman, at whose suggestion this work was undertaken, and under whose guidance it has been carried out.

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The Effect of the Concentration of the Nutrient Solution on the Growth of Barley and Wheat in Water Cultures.

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With Plate II and four Diagrams in the Text.

FOR some years past much discussion has taken place as to whether the concentration of the nutrient solution has any appreciable effect upon plant growth, and at the present time the controversy is far from settled.

Brezeale¹ carried out numerous water-culture experiments with wheat, using the transpiration as the criterion of growth. He states that 'it is evident that there is an optimum physical concentration of the nutritive solution at which water cultures of wheat thrive best, aside from variation in the amounts present of the different nutrient materials'. Cameron² interprets these results otherwise, and claims that Brezeale has shown that, 'in water-culture experiments with wheat, if a given ratio of mineral nutrients be maintained, relatively small effect is produced on the growing plants by varying the concentration over a wide range, in one case 75 parts per million to 750 parts per million, and this effect seems to be largely independent of the nature of the particular mixture of solutes'.

Hall and Underwood,³ however, obtained indications that with barley the concentration of the nutrient solution in water culture has a definite effect upon growth, the total dry weight of the plants decreasing with the strength of the solution. Recently Stiles⁴ has made further inquiry into the matter and states that 'the variation over a fairly wide range of the concentration of the nutrient solution of rye and barley growing in water cultures produces relatively little effect on the amounts of dry matter produced. Below a certain concentration there appears to be a definite falling off in the rate of growth.'

When the figures given by Stiles for the dry weights of barley in

¹ Brezeale, J. F.: Effect of the Concentration of the Nutrient Solution upon Wheat Cultures. *Science*, xxii, pp. 146-9 (1905).

² Cameron, F. K.: The Soil Solution, pp. 40-1.

³ Hall, A. D., Brenchley, W. E., and Underwood, L. E.: The Soil Solution and the Mineral Constituents of the Soil. *Phil. Trans. Roy. Soc.* 204, B. 307 (1913).

⁴ Stiles, W.: On the Relation between the Concentration of the Nutrient Solution and the Rate of Growth of Plants in Water Cultures. *Ann. Bot.*, vol. xxix (1915).

water cultures are compared with those for thousands of plants grown at Rothamsted during the last nine years, it is seen that they are remarkably low, so low as to suggest that some factor was in action at Leeds that was quite ignored or overlooked in the estimation of results. Plants are very sensitive to external influences other than those of food and water-supply, and the amount of light, variations of temperature, and the atmospheric conditions prevailing during the growing period all have definite action on the rate and quality of growth. Crowther and Ruston¹ have shown that the smoke pollution of the air at Leeds is so great that plant life is most seriously affected, considerable depression in growth being caused at the University. This factor must have operated upon the water cultures, and may, to some extent at least, have vitiated the results obtained. Whereas at Leeds in 1914 the mean dry weight of barley-plants grown from April 28 to June 6 was only 0.628 grm., at Rothamsted one series grown simultaneously from April 27 to June 9 averaged 2.516 grm. dry weight, another series averaging 2.252 grm., and this was in spite of the advantage gained by the Leeds plants in the frequent renewal of food solution, while the Rothamsted plants remained in the initial solution all through the experiment.

It has been stated that 'plants growing in water cultures under exactly the same conditions are very variable',² and this is used as an argument for discounting the value of water cultures as a method of experiment.³ As a matter of fact, the individual variation of plants within a single series is far less than with similar plants growing under natural conditions in the open field. It is only necessary to examine carefully a small area of barley in the field, plant by plant, and to compare with a number of water cultures growing at the same time, in order to be convinced of the truth of this fact. *Dactylis glomerata* is on the whole a bad subject for water-culture experiments, but even in this case the range of individual variation under such conditions is most obviously less than between plants growing on the experimental plots. Mean variation from series to series is fairly great, because the period of the year has a very great influence upon the rate of growth, and plants grown in January and February may possibly not reach one-quarter the development (as shown by dry weight) of similar plants grown in April and May for the same length of time. Experiments have shown that the difference of even a week in putting plants in water cultures has a distinct effect upon the total dry matter that can be produced within a given time. Every experimental method has its disadvantages and its weaknesses, and while water-culture methods are far from perfect, and indeed make no claim to be so, yet they do afford those conditions that are the most under the control

¹ Crowther and Ruston: Town Smoke and Plant Growth. Journ. Ag. Sci., vol. vi, Pt. iv, pp. 387-94.

² Stiles, W.: loc. cit., p. 89.

³ Stiles and Jorgensen: Studies in Permeability, I. Ann. Bot., vol. xxix, p. 349 (1915).

of the operator, and for that reason, if for no other, they have a special value of their own.

During the season of 1915 a number of water-culture experiments have been made to see if further light could be obtained as to the effect of varying concentrations of nutrient solutions upon growth, barley being used as the test plant in the three main series, wheat being grown in one case only. Four strengths of nutrient salts were used, N, N/5, N/10, N/20, the N-solution being that in general use in the laboratory, containing—

Potassium nitrate	1	gm.
Magnesium sulphate	0.5	"
Potassium di-hydrogen phosphate	0.5	"
Sodium chloride	0.5	"
Calcium sulphate	0.5	"
Ferric chloride	0.04	"
Distilled water	to make up one litre.	

The range of concentration was thus approximately 3,000, 600, 300, 150 parts of food-salts per million, containing potassium, phosphate, and nitrogen as in the following table:

<i>Parts per million of</i>	<i>Concentration of Solution</i>			
	N	N/5	N/10	N/20
K ₂ O	640	128	64	32
P ₂ O ₅	204	41	20.5	10.25
N	138	28	14	7

All the usual precautions were taken; the bottles were thoroughly washed, new corks were used in every case, the water was obtained from a silver still which was kept scrupulously clean and polished, and the food-salts were uniform all through the experiments and were weighed up separately for each unit of ten plants.¹ The barley was a pure strain of 'Plumage' obtained from Mr. Beaven, and the seeds were all graded between 0.05 and 0.06 gm. to reduce individuality as much as possible. The wheat was a pure line of 'Persian' wheat obtained through the kindness of Dr. N. Vavilov of Moscow; these seeds had to be sown without grading, as the supply was very limited.

In each experiment with barley 120 plants were grown in units of ten.

(1) All concentrations (N, N/5, N/10, N/20), the solutions being changed regularly every four days.

(2) All concentrations, the solutions being changed once, exactly half-way through the experiment.

(3) All concentrations, the solutions being never changed.

Great care was taken of the roots when the solutions were being changed. While the bottles were refilled, one by one, the plants were removed, and the roots laid in a saucer containing a little solution corre-

¹ The salts used were Kahlbaum's 'for analysis', and the stock was specially reserved for this experiment in view of the impossibility of replacing them at the present time.

sponding in strength to that from which the plant was taken, so that no check was caused, either by slight desiccation or by shock from the roots being laid in pure water or in an alien solution. Each test ran for seven weeks, and was repeated three times at intervals of three weeks, so that information was obtained for plants grown early and late in the season. The developmental history was carefully noted, and it was found that the difference in growth of plants in different concentrations was not only shown by the ultimate dry weights, but was apparent to the eye through the whole course of the experiments, both with regard to the size of plants and type of growth, especially with the roots. Each plant was harvested separately, and the dry weights of roots and shoots recorded.

First Series.

Seeds sown, March 5.

Plants put into solutions, March 15.

Plants harvested, May 3.

Solutions changed 'frequently', at regular intervals of four days.

Solutions changed 'once', on April 8.

Solutions frequently changed. Most of the plants started off with fairly normal root growth, but the $N/20^1$ began to vary within the first few days, remaining short, with short thin laterals, which gave the roots a square bunchy appearance compared to the usual long type. This 'bunchiness' persisted for several weeks, but eventually the laterals elongated more normally. An unusual feature of the root growth was seen in all concentrations at the end of about a month. In addition to the usual thin roots supplied with long thin laterals, there appeared a number of very thick long rootlets springing from the base of the plant, either entirely free from laterals or else furnished with a very few tiny ones. These rootlets were thickest and most numerous in the N-plants and persisted to the end, so that at harvest-time the roots were inclined to be thick and much less fibrous than usual. In the lower concentrations these roots were very prominent at the time of formation, but were overshadowed later on by the further development of fibrous rootlets, and at harvest the roots had regained a more normal type.

The development of the shoots in the plants growing in the different concentrations was very similar for some long time, but gradually a falling off was noticed with the two lowest ($N/10$, $N/20$), and by harvest-time some indications of this appeared even with $N/5$ shoots. In the N-plants the shoots were of an exceptionally dark green colour to the very end, the lowest leaves remained green and healthy, and there was no sign of red coloration at the base of the stem. The $N/5$ -plants showed similar

¹ For convenience of reference, the plants in the different concentrations will be called N, $N/5$, $N/10$, $N/20$ plants.

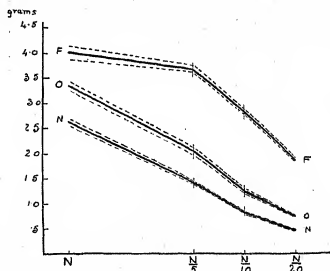
development, and were nearly as dark in colour when harvested, but some of the lowest leaves had begun to turn yellow, and a trace of red was visible in the stem a few days before cutting. In N/10-plants these phenomena were more marked. The withering of the lower leaves and coloration of the stem had set in at an earlier date and were more pronounced, also the general development was less good. With the lowest concentration (N/20) the shoots were very much smaller than in any of the others, and were of a yellowish green colour, while the lowest leaves had died off a fortnight earlier, at the same time as the red colour appeared in the stems.

The general trend of these observations is reflected in the dry weights of the plants, which will be discussed later.

Solutions never changed. The difference in concentration affected root growth immediately, each strength of solution having a definite effect of its own. The N/5-roots fell

behind the normal within a week, being short and rather bushy with laterals standing out from the rootlets at an angle. These laterals elongated later, and gave the roots a more typical appearance till they looked stronger than the N, but this appearance was falsified by the dry weights. In the lower concentrations the roots were very poor at first, bunchy, with rather thick laterals standing out on every side, giving the roots a 'stark' appearance, but later on development became more normal in type, though still weak. The abnormal development of thick unbranched rootlets seen with 'frequently changed' plants was not noticed in any instance where the solution remained unchanged throughout the course of the experiment. Shoot growth showed a regular depreciation as the concentration of the nutrient solution diminished. The weaker the solution, the earlier etiolation set in, and the sooner did the lower leaves begin to die off and the red colour appear at the base of the stem. Towards the close of the experiment the difference in the amounts of water lost by transpiration was very marked, hardly any being given off by the N/20-plants.

Solutions once changed. The single change of solution kept the N-plants growing better, so that at harvest-time the plants were more strongly developed and of better colour than in the 'never changed' set.



CURVE 1. Mean dry weights of ten barley-plants growing in nutrient solutions of different concentrations. Dotted lines show the limits of probable error. F, frequently changed; O, once changed; N, never changed. (March 15-May 3.)

82 *Brenchley*.—*Effect of Concentration of the Nutrient Solution*

With all other concentrations the march of events was delayed, but not arrested. Considerable improvement occurred immediately after the change (the weaker the solution the more obvious the improvement), but the falling off in growth soon reasserted itself in each case, though the 'fillip' caused by the renewal of the food supply was well reflected in the dry weights (*see* Table I and Curve I).

Series 1. March 15–May 3.

Solutions changed

<i>Solution.</i>	<i>Frequently.</i>			<i>Once.</i>			<i>Never.</i>		
	<i>Shoot.</i>	<i>Root.</i>	<i>Total.</i>	<i>Shoot.</i>	<i>Root.</i>	<i>Total.</i>	<i>Shoot.</i>	<i>Root.</i>	<i>Total.</i>
N	2.919	1.082	4.001 ± 0.142	2.639	0.713	3.352 ± 0.096	1.897	0.735	2.632 ± 0.071
N/5	2.608	1.041	3.649 ± 0.074	1.483	0.543	2.026 ± 0.072	0.958	0.446	1.404 ± 0.020
N/10	1.984	0.816	2.800 ± 0.049	0.791	0.447	1.238 ± 0.048	0.526	0.301	0.827 ± 0.015
N/20	1.303	0.528	1.831 ± 0.038	0.447	2.289	0.736 ± 0.001	0.244	0.208	0.452 ± 0.011

Series 2. April 5–May 24.

Solutions changed

<i>Solution.</i>	<i>Frequently.</i>			<i>Once.</i>			<i>Never.</i>		
	<i>Shoot.</i>	<i>Root.</i>	<i>Total.</i>	<i>Shoot.</i>	<i>Root.</i>	<i>Total.</i>	<i>Shoot.</i>	<i>Root.</i>	<i>Total.</i>
N	4.442	1.330	5.772 ± 0.193	3.746	1.324	5.070 ± 0.209	3.048	1.159	4.207 ± 0.113
N/5	3.365	1.137	4.502 ± 0.076	1.621	0.709	2.330 ± 0.117	1.136	0.631	1.767 ± 0.037
N/10	1.899	0.843	2.742 ± 0.097	0.841	0.470	1.311 ± 0.056	0.525	0.409	0.934 ± 0.040
N/20	1.283	0.683	1.966 ± 0.042	0.363	0.318	0.681 ± 0.025	0.253	0.258	0.511 ± 0.015

Series 3. April 26–June 14.

Solutions changed

<i>Solution.</i>	<i>Frequently.</i>			<i>Once.</i>			<i>Never.</i>		
	<i>Shoot.</i>	<i>Root.</i>	<i>Total.</i>	<i>Shoot.</i>	<i>Root.</i>	<i>Total.</i>	<i>Shoot.</i>	<i>Root.</i>	<i>Total.</i>
N	5.137	1.320	6.457 ± 0.144	3.218	1.400	4.618 ± 0.117	2.401	1.293	3.694 ± 0.101
N/5	4.574	1.467	6.041 ± 0.124	1.841	0.804	2.645 ± 0.067	0.922	0.690	1.612 ± 0.080
N/10	2.286	0.806	3.092 ± 0.175	0.428	0.220	0.648 ± 0.032	0.285	0.233	0.518 ± 0.011
N/20	1.254	0.527	1.781 ± 0.074	0.179	0.113	0.292 ± 0.010	0.085	0.081	0.166 ± 0.015

Table I. Mean dry weights in grams of ten barley-plants grown in nutrient solutions of various strengths.

Second Series.

Seeds sown, March 27.

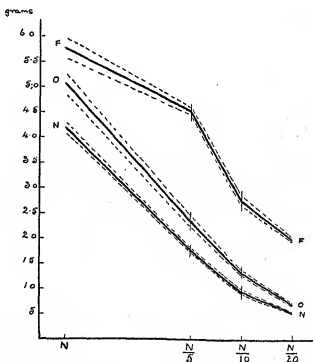
Plants put in solutions, April 5.

Plants harvested, May 24 (Plate II, Figs. 1, 2, 3).

Solutions changed 'frequently', at intervals of four days.

Solutions changed 'once', on April 30.

The course of events was the same as in the first series, though growth was more rapid owing to the more favourable season for growth, and differences due to the varying concentrations were more marked than in the earlier experiment (Curve 2). The thick rootlets in 'frequently changed' plants were less strongly developed. It seems probable that the frequent renewal of the nutrient salts caused the plants to put out the abnormal rootlets for some unexplained reason, particularly at the time of year when growth was fairly slow. When growth was more rapid, the root development remained more normal in type, though thick rootlets did appear to some extent. Later on in the year they were only produced by plants in high concentrations, the others bearing quite normal fibrous roots. It may very tentatively be suggested that the thickened rootlets provide a means of protection at certain periods of growth against the constant change of balance due to the frequent renewal of the food solution. As they are so badly provided with laterals, it may be that they are able to prevent the ingress of too great and sudden an influx of food material at the time of the change of solution, so that they act as a kind of control. When growth is more rapid, the plant can deal with extra food more readily, so that the controlling function is of less importance, and the thick roots are correspondingly less developed. This idea is also borne out by the fact that in the first series the N-plants produced very thick rootlets in quantity, rendering the root thick and much less fibrous than usual, thus indicating possibly that at that time of year the plant was never able fully to cope with such a constant renewal of food solution of high concentration, owing to the relative slowness of growth which entailed the utilization of a lesser quantity of plant food (see Table I).



CURVE 2. Mean dry weights of ten barley-plants growing in nutrient solutions of different concentrations. Dotted lines show limits of probable error. (April 5-May 24.)

Third Series.

Seeds sown, April 16.

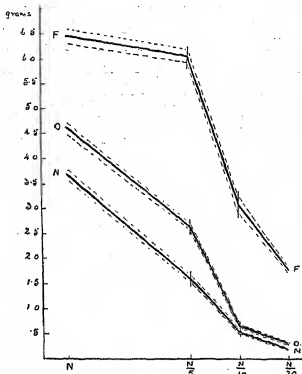
Plants put into solutions, April 26.

Plants harvested, June 14.

Solutions changed 'frequently', every four days.

Solutions changed 'once', May 20.

This series was started rather late in the season, so that in some ways the plants exhibited more variability than in the earlier experiments. With



CURVE 3. Mean dry weights of ten barley-plants grown in nutrient solutions of different concentrations. Dotted lines show limits of probable error. (April 26-June 14.)

after which very long thin roots were produced. The shoots were also long and very thin, with little or no tillering. The sharp falling off in growth below the N/5 concentration, even when the solutions were changed, is well shown in the graph (Curve 3 and Table I).

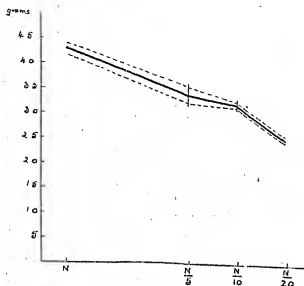
DISCUSSION OF RESULTS.

An examination of the figures and curves of dry weight in all three series shows that, however the solutions are treated, there is a steady decrease in the dry weights of the plants as the strength of the nutrient solution gets less. This decrease in weight is very considerable and is always outside the limits of experimental error. The results run in the same direction in all the experiments, the differences being accentuated in the sets grown later in the year, when growth is more rapid. It is noticeable that the drop in dry weight from N to N/5 is far less when the solutions are changed frequently,

frequently changed solutions the life-histories were much as usual; in the N-plants the roots were inclined to be rather short and bushy from first to last, and were well supplied with the typical thick rootlets; for about a month the N/5-plants looked stronger than the N, but this did not continue; in lower concentrations a rapid falling off of growth was exhibited. The red coloration did not appear in the stems, but the lower leaves were dead in every case. When the solutions were rarely or never changed, the plants in the two lower concentrations made little or no headway for a long time. Root development was checked almost entirely for three or four weeks,

and in some cases (Series 1 and 3) the approximation is fairly close. This suggests the possibility that more frequent renewal of the solutions, maintaining more evenly the balance of the nutrient salts, would be followed by as much growth in the $N/5$ as in the N concentration, although the tendency is for growth to fall behind in the lower strength with small provocation. In other words, if it were possible to arrange an experiment in which the balance of the nutrient solution was kept constant by the automatic replacement of the food-salts absorbed, it is conceivable that plants in these two concentrations might produce equal quantities of dry matter. But there are indications that toxic effects would set in under these circumstances in the N solution, as some of the constituents might be present in so great a quantity as actually to put a brake on plant growth. In the $N/5$ solution, on the other hand, the probability of such action is considerably less, and the plants would continue to make full use of the food-salts and would approximate in growth to those in the N solution. If this supposition be correct it is not true to say that the plant is indifferent to the variation in the strength of these two solutions, but that it responds to increased strength by increased growth. With the highest concentration, N , however, another factor, that of toxic action, comes into play, counterbalancing the increased growth and reducing it to the level of that attained with the lower ($N/5$) concentration. Further experiments are being made to obtain more definite information on this point, and also to find out whether there is an optimum concentration for growth or whether the plant will grow equally well within a certain range of the higher concentrations. The main point at issue at present is not that of equal growth in varying concentrations, but that of the great dilution at which it is claimed that such equal growth can be made. With concentrations below $N/5$ a very different result is obtained. The more frequently the solutions are changed, i. e. the closer the concentrations approach to a state of constant balance, the more marked is the drop in the dry weights as the strength of the solution decreases. This implies that with the lower strengths the plant is living in a condition of semi-starvation. When the solutions are changed frequently, the improvement of growth is the more marked the higher the concentration (up to a certain limit, $N/5$ in this experiment), owing to the sum total of food supplied approximating more closely to the needs of the plant for optimum growth. It is difficult to imagine that, even if a constant balance of food-salts could be maintained, the plants in the solutions below $N/5$ would in any way approach those in the higher strengths, as, if this were indeed the truth, some indications of it would have been obtained by some incipient approximation of dry weights, such as occurred with plants in N and $N/5$ solutions, instead of a marked and decreasing divergence of these weights as the concentrations rose towards $N/5$, when solutions were frequently renewed. As a matter of fact, the actual dry weights of the $N/10$

and N/20 plants in frequently renewed solutions were practically the same, within the limits of error, in all three experiments, showing that the same amount of growth had taken place in each case, whereas normally plants grown later in the season form much more dry matter, owing to the increase in the rate of growth, the N and N/5 plants in the three series being about 50 per cent. heavier than those in the first series grown seven weeks earlier.



CURVE 4. Mean dry weights of six wheat-plants grown in nutrient solutions of different concentrations. Dotted lines show limits of probable error. Solutions frequently renewed. (March 16–May 11.)

This indicates that with the lower strengths the amount of growth was strictly limited by the quantity of food supplied, and that it was impossible for the plants to reach full development with such a restricted amount.

In the single solution with wheat, the solution was frequently changed, and the plants were grown on for eight weeks. In this case again, a steady fall in dry weight occurred with decrease of concentration, but owing to lack of seeds it was not possible to carry the comparison so far as with barley. The drop is less marked with wheat, but this may

be because it grows more slowly than barley, so that differences are less accentuated within the same limits of time, though they are none the less definitely marked (Curve 4 and Plate II, Fig. 4).

Before proceeding to discuss the significance of these results further, it may be useful to summarize the effect of varying concentration of food-salts obtained by different workers.

Worker.	Plant.	Parts per million of Food-salts.
Brezeale	Wheat	750–75 growth similar.
Stiles	Barley	1,800 360 180 90 growth similar. shows slight decrease.
Hall and Underwood . . .	Barley	3,000 600 300 150 steady decrease in growth.
Brenchley	Barley	3,000 600 300 150 growth possibly much same in growth (solutions changed). marked decrease in growth (solutions not changed).
Brenchley	Wheat	3,000 600 300 150 steady decrease in growth (solutions changed).

The above table shows how great are the discrepancies between the results of several workers dealing with the same species by the same method of water-culture experiments. In the explicit statement by Cameron quoted earlier, it is claimed that the effect of the varying concentrations is largely independent of the particular mixture of solutes. If this statement be true, it matters little what nutrient solution is used, provided plants will make good growth in it, and the argument cannot be advanced that the difference in *composition* of the solution explains the discrepancies between the results, *concentration* being the only point at issue, provided that balance or a given ratio of nutrient salts be maintained as far as possible. Stiles¹ probably came very close to the truth in saying that 'possibly in the American experiments something other than concentration of salts was acting as a limiting factor in all cases', but apparently he failed to see that the same remark may have had a very pertinent bearing upon his own results, owing possibly to the smoke factor in the Leeds district. Brezeale found with wheat that about 300 parts per million of food-salts gave maximum growth, and that growth fell off as the concentration increased to 750 parts per million or decreased to 75 parts per million, whereas at Rothamsted wheat shows a steady depreciation of growth as the concentration decreases from 600 to 150, well within the former range. Stiles maintains that barley grows equally well within the range of 1,800 to 180 pts. per mil., and only shows a slight depression with as little as 90 pts. per mil., whereas at Rothamsted a great and marked decrease in growth occurs from 600 to 150 pts. per mil. It seems more than probable that some depressing factor must have been at work in the Leeds experiments, which tended to equalize the growth of the plants by hindering them in some way which prevented normal development and reduced growth to a dead level. Such a factor might be provided by the presence of minute quantities of a toxic body in the distilled water or in the salts used for making up nutrient solutions. Experience has shown that the presence of the merest traces of copper salts in differential experiments will vitiate growth to such an extent as to make comparison useless, and unless the water is prepared with the utmost care such toxic substances find only too easy an entrance. Unfavourable atmospheric conditions, unsuitable temperature, lack of cleanliness in working, growth of algae in culture bottles, and the admittance of light to the roots are a few of the factors which may adversely affect growth, and which have to be taken into consideration in estimating results if they come into play. It is impossible to generalize from water cultures to sand or soil cultures, or from one species to another, but so far as the growth of barley and wheat in water cultures is concerned, this last experiment at Rothamsted upholds the earlier contention of Hall and Underwood that the concentration of the nutrient solution influences very

¹ Stiles, W. : Review, *Journ. Ecol.*, vol. ii, p. 54 (1914).

greatly the rate of growth of plants. Not only is the *rate* of growth affected, but the *amount* of growth is strictly limited by the quantity of available food when the nutrient solutions are dilute. Little work has yet been done with higher concentrations, but it is possible (see page 85) that toxic action due to over-nutrition from too great a supply of food-salts comes in to counter-balance or replace the increase of growth caused by increase of nutriment which occurs with lower strengths.

Although the experiments fail to corroborate the idea that concentration is unimportant within very wide limits, still they fully support other observations made by Stiles. In every case there is a drop in the dry weight of plants grown in any concentration according to the frequency with which the solutions are changed, the 'frequently changed' plants being heavier than the 'once changed', and the 'once changed' than the 'never changed'. With the normal strength it is probable that there is a sufficient supply of food material even when no renewal of solution takes place. In one case, barley was grown in such a normal solution for over eight weeks, and analyses made at the end of the experiment showed that 25 per cent. of the initial nitrogen still remained in the solution, and as the nitrogen compounds are absorbed in greater quantities than other salts it is evident that an ample sufficiency remained, if the quantity of the salts were the only factor concerned. Thus it is probable that with this concentration the question of starvation does not arise, and that the steady decrease in weight is really associated with the change of balance of the nutrient salts, the plants being the better the closer the initial balance is maintained.

With the lower concentrations, the drop in weight from 'frequently changed' plants to the others was much heavier. Since with the normal solution the decrease in weight due to the balance of the food-salts was so much less marked, it seems permissible to assume that the very heavy drop with the lower concentrations is due largely to quite another cause, that of varying degrees of starvation through lack of sufficient nutriment. When the solutions are changed as often as once in four days, twelve times altogether, the plant has access during its lifetime to a far greater store of food material than when solutions are seldom or never changed. Consequently such plants suffer less from the starving effects due to the low concentrations of the food-salts in solution, but still the response corresponds strictly to the amount of food available at any one time. Therefore it seems evident that with the normal solution the change in the balance of food-salts has a hindering action upon the growth of barley, and that this hindrance is coupled with varying degrees of starvation as the concentration decreases, being specially accentuated in those cases in which the solution is never changed.

It has frequently been noted that the variation in the strength of the food solution not only affects the total dry weight of the plant, but

also has a very marked action upon the relative rate of growth of roots and shoots, and this is well shown in the experiments under discussion. All the way through, the shoot responds more sharply than the root to the changes in food supply; consequently, as the solution decreases in strength the ratio between the dry weights of shoots and roots also decreases; in other words, the weights of the roots and shoots tend to approximate more closely as the supply of nutriment gets smaller, until in some cases with very dilute solutions the root is as heavy as, or even heavier than, the shoot (Table II). The change in ratio takes place always, whether the solutions are changed or not during growth, but it is most marked in those cases in which great starvation has ensued owing to the low concentrations not being renewed. It seems as though the plant makes every endeavour to supply itself with adequate nutriment, and as if, when the food supply is low, it strives to make as much root growth as possible so as to offer the greatest absorbing surface for whatever nutriment may be available.

Shoot/Root Ratio.

Series 1. March 15-May 3.

	N	N/5	N/10	N/20
Solutions changed frequently . .	2.691	2.506	2.431	2.468
" " once . . .	3.702	2.731	1.770	1.548
" " never . . .	2.580	2.149	1.750	1.174

Series 2. April 5-May 24.

	N	N/5	N/10	N/20
Solutions changed frequently . .	33.41	2.956	2.252	1.876
" " once . . .	2.830	2.288	1.791	1.144
" " never . . .	2.630	1.800	1.285	0.979

Series 3. April 26-June 14.

	N	N/5	N/10	N/20
Solutions changed frequently . .	3.892	3.117	2.835	2.379
" " once . . .	2.297	2.289	1.946	1.582
" " never . . .	1.857	1.336	1.226	1.039

Table II. Showing the ratio between the shoots and roots of the barley-plants whose mean dry weights are given in Table I.

SUMMARY.

When plants, such as barley and wheat, are grown in water cultures under favourable conditions, the concentration of the nutrient solution, up to a comparatively high strength, has a great effect upon the rate and amount of growth, even when the balance of the solution approximates to a constant level. Starvation effects, due to insufficient nutriment, are evident in much stronger concentrations than has been admitted by some

other observers. The action of different high concentrations of constant balance has not yet been determined, and it is uncertain whether there is an optimum strength, above and below which growth falls off, or whether there is a range of concentrations between which the plants will make equally good growth. It seems evident, however, that if water cultures with wheat and barley are carried out under advantageous growth-conditions, complete and maximum growth cannot be obtained in a solution containing the amount of potash and phosphoric acid (K_2O 28 p.p.m., P_2O_5 7 p.p.m.) stated by Cameron to exist in the soil solution.

1 ROTHAMSTED,
October, 1915.

EXPLANATION OF PLATE II.

Illustrating Dr. Brenchley's paper on *Concentration of Water Cultures*.

Fig. 1. Photograph showing the growth of barley-plants in water cultures when solutions were frequently renewed. Concentration of solutions, N, N/5, N/10, N/20. Series II.

Fig. 2. As above, but solutions changed once only. Series II.

Fig. 3. As above, but solutions never changed. Series II.

Fig. 4. Photograph showing the growth of wheat-plants in water cultures when solutions were frequently renewed. Concentration of solutions, N, N/5, N/10, N/20.

The Origin of the Endodermis in the Stem of *Hippuris*.

BY

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With six Figures in the Text.

THE question of the three germinal layers exhibited by the stem and root of Dicotyledons is a very old one, and has been the subject of investigation by many botanists. The idea that these three developing layers were the initials of definite tracts of tissue of the adult plant was first put forward by Hanstein. He supposed that of these three layers or histogens, the dermatogen gave rise to the epidermis, the periblem to the cortex, and the plerome to the central cylinder.

The fact that in the adult organs the innermost layer of the parenchymatous tissue surrounding the central cylinder is frequently differentiated by special characters has given to this layer, the endodermis, a special significance. The reason that it has acquired so much importance is that it has generally been regarded as the innermost layer of the cortex and developed from the innermost layer of the periblem, forming thus the boundary of the stele.

The importance of the endodermis as a morphological unit thus obviously depends on the uniformity of its mode of origin. This was realized by Schoute (1), who in 1902 published a general review of the stelar theory, and incidentally made a critical examination of a number of stems and roots. He showed that the majority of species examined exhibited a well-marked endodermis. The origin of this layer from the apical tissue of the stem was investigated in the case of only six species, in some of which no distinction between periblem and plerome could be made out. Among the plants examined was *Hippuris vulgaris*, in the stem of which the apical structure is so clear that it has become a classical type for use in the laboratory.

Schoute, using series of transverse and longitudinal sections, traced the history of the development of the tissues from the apex to the mature part of the plant, and was led to the conclusion that here not only the endodermis, but several other layers of the cortex were derived from the plerome.

This observation was directly opposed to the generally accepted idea of the origin of the tissues of *Hippuris* put forward by Sanio (2), according to which the endodermis was derived from the innermost periblem layer.

Since Schoute's observation has so far received no independent confirmation, a re-examination of the critical case of *Hippuris* seemed desirable, and material was therefore collected in the spring of 1914 and 1915. Schoute's methods of investigation were closely followed. Stem apices were selected from shoots of various sizes, care being expended to choose only straight ones. These were embedded and cut in series of transverse sections up to within a distance of about 100μ from the apex. The block was then rotated at right angles, and the remainder of the apex cut in a longitudinal series.

The advantage of this method is apparent. A median longitudinal section through the apex shows very clearly the distinction between the periblem and plerome, which is made evident by the small number and extreme regularity of the layers of periblem cells which form a series of caps covering the central column of plerome, in which the cells are less regularly arranged. In transverse sections the distinction is not so clear in this region, and the use therefore of longitudinal sections through the extreme apex of a stem of which the lower part is cut transversely, enables one to determine the exact number of periblem layers concerned in the origin of the mature tissues.

It is thus possible to trace with certainty the critical layer, the innermost of the periblem, and to identify the structure derived from it.

In order to be quite certain about the position of the delicate cell-walls of the developing tissues, it is necessary to clear away the cell-contents, and this can only be done satisfactorily by the use of eau de javelle on the sections. The clearing agents failed to penetrate the fixed material in bulk, even after the lapse of several days; on the other hand, the fresh material, though sufficiently cleared, was unsatisfactory because the cell-walls were also affected, losing their original firm contour, and in some cases actually breaking down. In order to be able to use eau de javelle on serial sections, it was necessary to employ a fixative other than albumen; water alone, collodion, and collodion with clove oil were tried, and a mixture of the latter in the proportion of 1 : 3 was found most satisfactory. With this fixative it is necessary, first, to float out the sections on water and then to transfer them to a slide smeared with the mixture. It is essential to have absolutely clean slides and to use fixative recently made up.

Of the many different stains employed, polychrome methylene blue gave the best results. It is, however, difficult to keep sufficient stain in the sections owing to its tendency to wash out in alcohol, unless the sections are mordanted after staining by placing them for a few minutes in a 10 per cent. solution of ammonium molybdate.

The advantages of polychrome methylene blue are twofold ; it is a very quick and easily controlled stain, and it differentiates well. The walls of the cells lining the air-passages stain differently from the others, and are thereby easily distinguished even in longitudinal sections.

The structure of the apex of the stem as seen in median longitudinal section (Fig. 1) exhibits an arrangement of cell-layers which follows diagrammatically the scheme set forth for stem apices in general. The outside is clothed by a single layer of regularly arranged cells, which constitutes a typical dermatogen. Within this, in the specimen figured, are five regular layers of cells forming the periblem. The cells of these differ little in form from those of the dermatogen. Within these, the cells of the plerome are more irregularly arranged, owing to the fact that they divide by both periclinal and anticlinal walls.

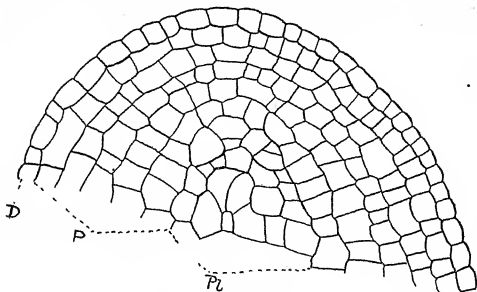


FIG. 1. Longitudinal section of tip of stem of *Hippuris vulgaris*. *d*, dermatogen ; *p*, periblem ; *pl*, plerome.

There is considerable variation in the size of the stem apices, and the number of periblem caps in the material examined varies from three to six. The general arrangement in other respects is quite consistent.

On turning to the transverse series and examining the first section (Fig. 2) it is a simple matter to interpret the layers of cells in terms of the longitudinal section. In some cases there is more difference between the size of cells of the periblem and those of the plerome than appears in the specimen figured. This difference becomes more pronounced as the sections are traced further down the stem. The cells of the plerome at an early stage divide rapidly by walls in all directions, thus producing a large number of small cells. The method of division is fairly regular. One cell divides into two, and these again divide independently by walls nearly at right angles, thus forming groups of four cells. These groups frequently

continue segmenting, but it is generally possible, for quite a long time, to trace the outlines of these groups which originate from separate initials. The cells of the periblem do not divide so early, but enlarge to keep pace with the increasing bulk of plerome, and later divide by anticlinal walls, thus preserving the original number of layers.

The air-channels begin to form in the periblem as intercellular spaces at the angles between cells of different layers. As a result, one finds rings of small air-spaces appearing between successive layers of periblem cells.

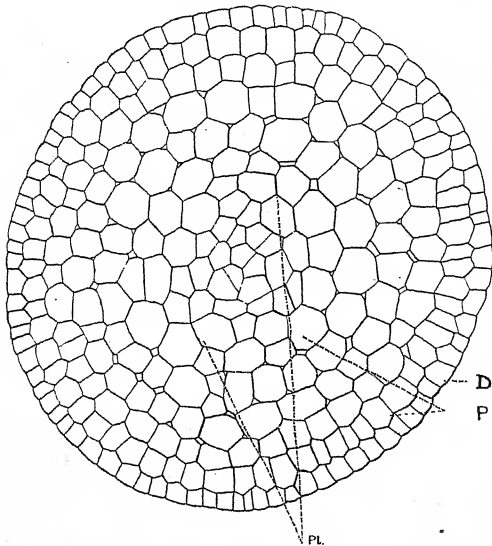


FIG. 2. Transverse section of same tip at region where the longitudinal section ends.

Such spaces are never found between the dermatogen and the first layer of the periblem; moreover, the ring of canals between the first and second periblem layers develops a little later than the ones within. As can be seen by reference to Fig. 3, there are four rings of canals developing.

At this stage the cells of the periblem begin to divide, and the subsequent arrangement of the cortical cells and air-canals is dependent on the direction in which these periblem cells segment. The innermost layer becomes divided by periclinal walls which are laid down in such a way as to

abut upon an air-space at either end. The relative position of the original cells is preserved, as very little growth of the daughter-cells takes place for some time subsequent to the division (Fig. 3). In the outer layers the original cells may undergo subdivision into two or three, rarely more. The walls may be laid down in any direction, which is however determined by the position of neighbouring air-canals, since the ends of these walls invariably abut upon these passages. Thus at this stage every intercellular space is a centre from which a varying number of walls radiate (Figs. 3, 4). With the increase in size of the stem which now follows, the cells of the develop-

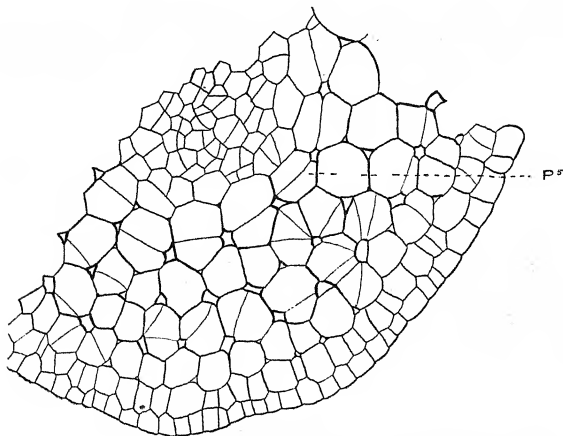


FIG. 3. Transverse section showing the innermost layer of periblem after the division into inner and outer cells. P^s , innermost periblem layer.

ing cortex undergo further subdivision, whilst at the same time the intercellular spaces enlarge enormously, owing to the rapid growth of these cells which surround, and eventually form single chains of cells separating them.

The development of the system of intercellular spaces just described serves to differentiate, very markedly in the young stem, the nodes and internodes, since the air-canals are only developed in the latter.

In the young nodes, on the other hand, cell-division takes place to a greater extent than in the internodes, first in connexion with the formation of the leaf-rudiments, secondly with the laying down of the pro-

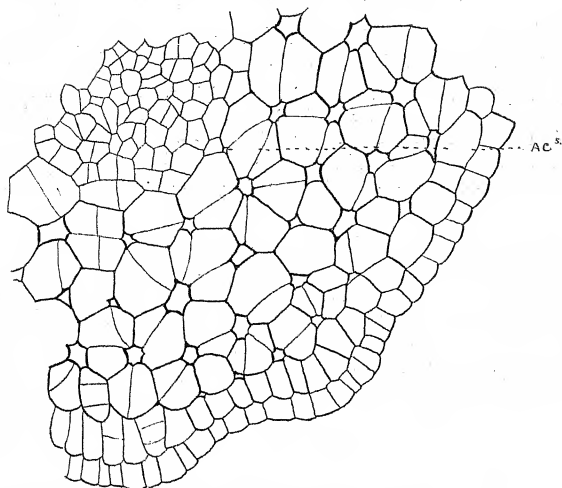


FIG. 4. Transverse section showing the further division of the perilem cells and the first appearance of the fifth ring of air-canals (*ac⁵*).

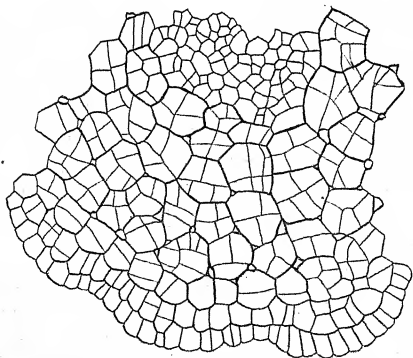


FIG. 5. Transverse section in the region of a node, about the same level as Fig. 4.

cambial strands of the leaf-traces, and thirdly to keep pace with the rapid increase in size of the young cortex consequent upon the formation of the air-canals in the internodes. For this reason the identity of the original periblem caps and their constituent cells is more difficult to trace in the node, though the contour of the original cells can as a rule be recognized by the thickness of their walls (see Fig. 5).

It has already been indicated that the number of circles of air-canals is related to the number of layers of periblem, being one fewer; thus, in the specimen figured with five periblem caps there are four rings of canals (Fig. 3). In the older parts of the stem, however, additional rings can be observed, and it is in studying the modes of formation of these additional intercellular spaces that the part played by the plerome in contributing to the inner region of the cortex, including the endodermis, becomes clear.

It is necessary therefore to return and to consider in some detail the fate of the most internal layer of the periblem. As described above, the first dividing-wall runs tangentially across the cell from one air-canal to another (Fig. 3). Further segmentation takes place in the inner and larger of the two cells so formed, and in this the next wall is placed in an obliquely radial direction, with its outer end usually based upon an air-canal. Where the inner end of this wall intersects the wall of the outermost plerome cell a new intercellular space arises, and thus is initiated a fifth ring of air-canals (Fig. 4, *ac.*⁵). It is obvious that cells bounding these canals on the inner side must be derived from the plerome, and this can be readily confirmed. Moreover, it not infrequently happens that in the development of the fourth ring of air-canals the separating cell-walls of the two cells bounding it on its inner side may split apart, thus permitting the enlargement of a plerome cell which thus becomes the inner boundary of the canal. The whole plerome is at this stage actively enlarging, but the outermost cells divide for the most part by radial walls, and also increase in size in a tangential direction. They thus form a fairly well defined layer distinct from the rest of the plerome. Although no regular sequence can be traced in their divisions, they sooner or later divide by tangential walls into inner and outer cells (Fig. 6). This stage can be traced for some distance down the stem. Both layers may undergo subdivision by radial walls. The cells of the outer layer eventually enlarge and become rounded off as intercellular spaces develop between them. Eventually the cells of the inner layer undergo a tangential division, and the relative position of the two layers of cells so formed remains unaltered in the mature stem. The innermost layer is the endodermis, and later develops the cuticularized folded band on the radial walls.

Considerable variation may be seen from the process just described. Some of the divisions may be omitted or, in other cases, may be increased in number, but on the whole the general sequence of development in

the outer part of the plerome is that described above, and thus normally three layers of the inner cortex, including the endodermis, take their origin from the outer plerome.

This conclusion agrees with that of Schoute, and is thus directly opposed to the earlier explanation given by Sanio, who considered that the

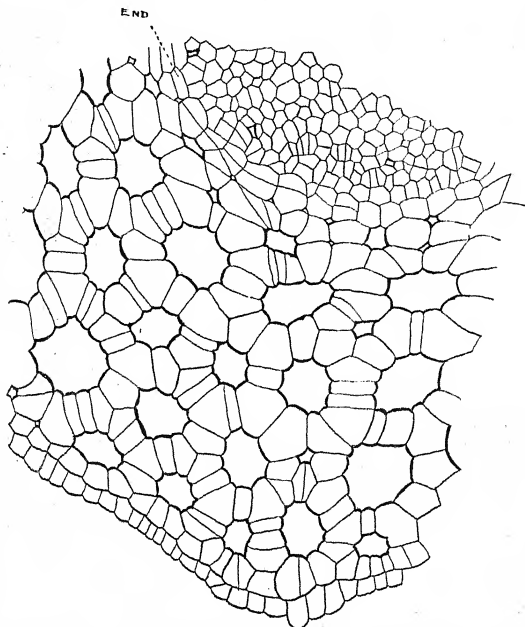


FIG. 6. Transverse section through older stem, showing the formation of the endodermis and final appearance of air-canals. *end.*, endodermis.

three layers in question originated by the subdivision of the innermost layer of the periblem.

Although the observations do not coincide in exact detail with those of Schoute, this is probably to be accounted for by the fact that, owing to this investigation having been confined to this one species, it was possible to examine a large number of apices and thus to obviate the disadvantages

connected with a description of one particular specimen. There is a wide variation in dimensions of the stem at the apex which is associated with differences in the number of periblem caps and bulk of the plerome. The number of the former may even vary in one and the same apex, as, for instance, in one specimen examined there were five caps on the one side of the stem and four on the other. The results of this investigation therefore confirm the observations of Schoute, and emphasize his conclusion that the endodermis cannot be regarded as a layer of definite morphological value. That is to say, its ontogenetic history is shown to be variable as elucidated by a study of cell-lineage. The only criterion of morphological identity in such instances would have to reside in the structure of the completely differentiated tissues, irrespective of their cellular history. This was, speaking generally, the position adopted by Van Tieghem, and whilst it illustrates the plasticity of the plant cells in differentiating into this or that form of tissue, it emphasizes the abstract character of the so-called morphology of the tissues themselves.

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1. SCHOUTE, J. C.: Die Stelar-Theorie. P. Noordhoff, Groningen, 1902.
2. SANIO, C.: Ueber endogene Gefäßbündelbildung. Bot. Zeit., xxii Jahrgang, 1864.

Fig.2

SOLUTIONS CHANGED ONCE

1 foot

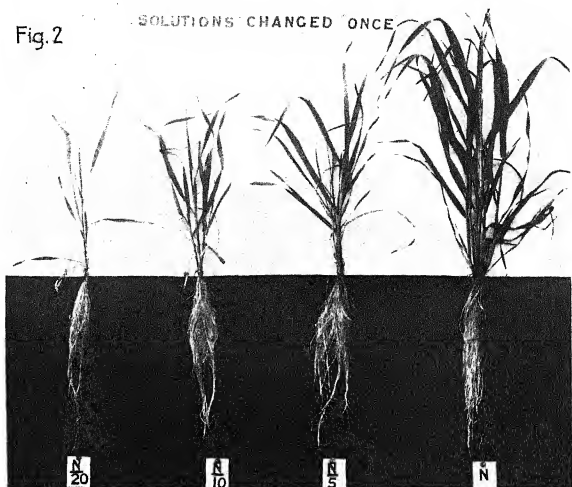


Fig.1

SOLUTIONS
CHANGED
FREQUENTLY



BRENCHLEY — WATER CULTURES.

Fig. 3

SOLUTIONS "NEVER CHANGED"

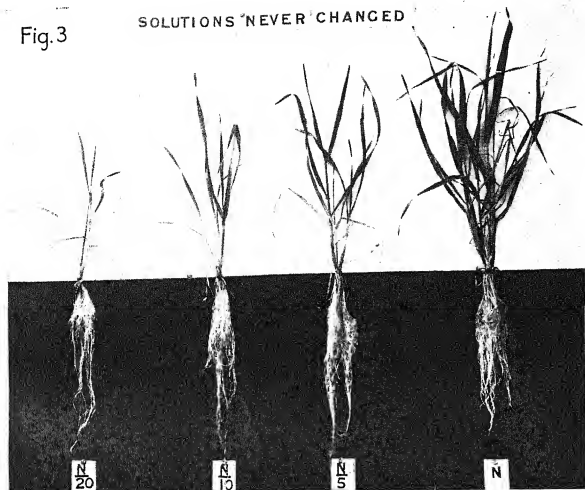
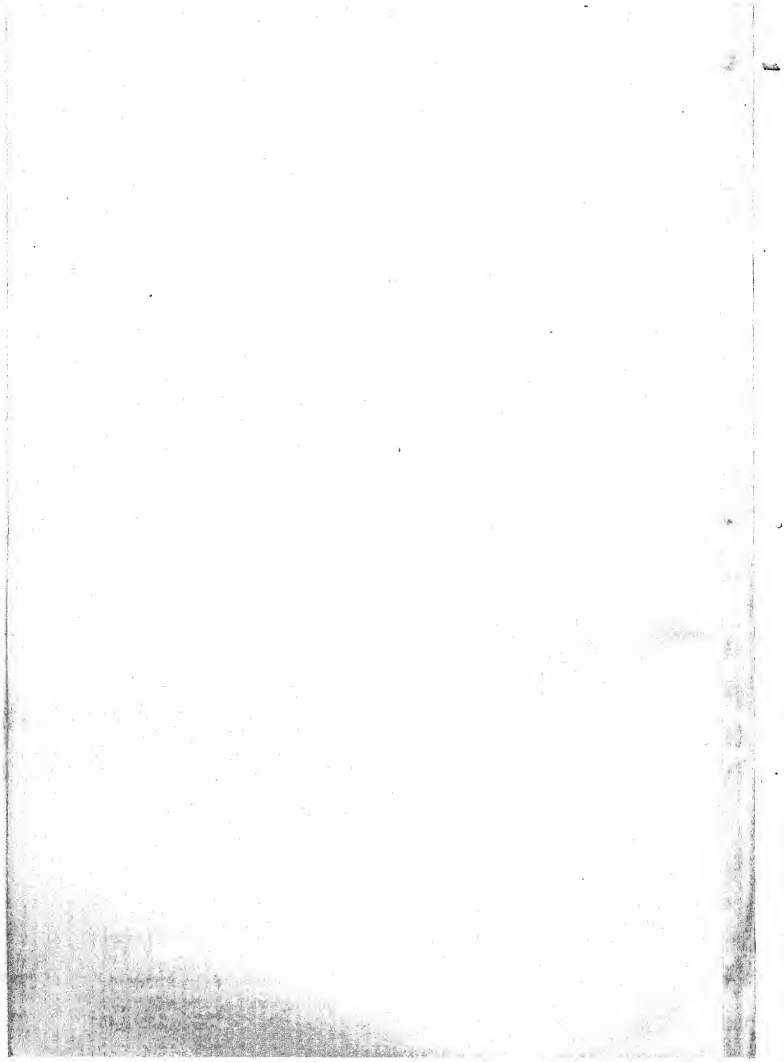


Fig. 4

SOLUTIONS*
FREQUENTLY
CHANGED





The Development of the Sorus and Sporangium and the Prothallus of *Peranema cyatheoides*, D. Don.

BY

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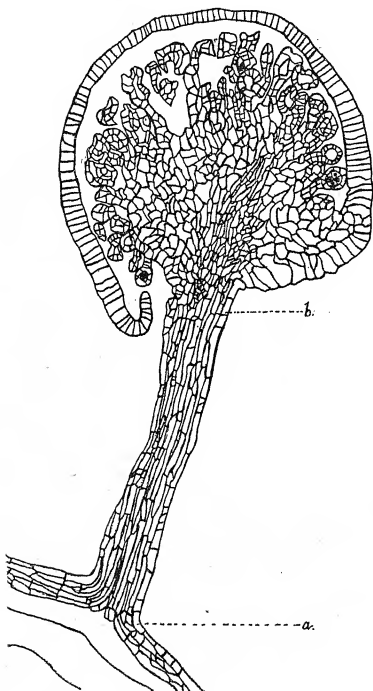
With Plate III and two Figures in the Text.

IN a paper in the *Annals of Botany*, vol. xxvi, 1912, it was suggested that *Peranema cyatheoides*, D. Don, occupied a position intermediate between the Cyatheaceae and the Aspidieae group of Polypodiaceae. Various features of the mature plant, including those of the vascular system and the sporangium, suggested a relationship with the species of *Nephrodium* and especially with *N. Filix-mas*, Rich. Developmental stages of the sorus and sporangium have since been studied in material grown in the Glasgow Botanic Garden and kindly forwarded to me by Professor F. O. Bower, F.R.S. Fresh spores were sent from India through the kindness of the Director of the Calcutta Botanic Garden, and young plants were reared from them by Mr. L. B. Stewart, Plant Propagator in the Royal Botanic Garden, Edinburgh. In the Edinburgh Garden there are now half a dozen strong and healthy plants of *Peranema*. I tender my thanks to the gentlemen to whose courtesy this result is due.

DEVELOPMENT OF THE SORUS.

From the earliest stages available it appears that the sorus is very soon after its appearance covered almost completely by the indusium (Pl. III, Fig. 1). This indusium is composed, in the main, of a single series of cells though near the highest point of its curve and near its tip there are two cell-layers. It forms a scale attached at one side along a semicircular line to the under surface of the leaf and bent over the top of the receptacle. At one point the receptacle is, for a width of three cells, uncovered by the indusium. Below this point are one or two cells continuous with the series forming the epidermis of the leaf and with walls thickened like those of the cells of the indusium (Fig. 1). This suggests the presence of a small second flap. Sections through older sori confirm the suggestion, since in these a distinct small second flap or edge of a cup is present (Figs. 2 and 3). In Fig. 3 the further curving over of the main indusial flap is shown. This is still more accentuated in Fig. 4, where the commencement of an extension of the receptacle at right angles to the leaf-surface can be distinguished. The second flap

still is present as the sorus lengthens (Fig. 5). When the receptacle grows out further as the narrow stalk of the sorus, the curving over of the indusial flap is very marked. It is then curved back on one side, until the portion



TEXT-FIG. 1. Vertical section through a semi-mature sorus, showing the 'stalk' fully developed. The main indusial flap now completely covers the wide receptacle and is incurved at its tip. The small knob opposite to this incurved tip represents the remains of the laggard portion of the indusium. The 'kink' shown in Pl. III, Fig. 4 has developed as the series of cells on the right of the sorus and now extends almost at right angles to the 'stalk'. The youngest sporangia occur among the stalks of the oldest at the summit of the receptacle; quite young sporangia appear at its margins. The sequence of sporangia is thus at first basipetal, and later mixed. $\times 65$.

by which it is attached to the stalk is almost parallel to the surface of the leaf (Text-fig. 1). The earliest indications of this recurving at the side are seen in Fig. 4.

Of the second flap there remains in the older sorus only a mere knob, seen on the left side of Text-fig. 1, at the top of the stalk. The extreme tip of the indusium is bent in (Text-fig. 1), and between the recurved tip and the stalk is a narrow slit, which appears on the surface of the sorus as an elongated pore. This slit marks the edge of the 'cup' forming the indusium. In a transverse or oblique section of the mature sorus the two edges of the 'cup' may be seen (cf. Pl. III, Fig. 6, and Ann. of Bot., xxvi, Pl. XXIX, Fig. 15).

These sections through the sorus show that the indusium arises as a cup round the receptacle, that one side of the cup is strongly developed and curved over the top of the receptacle, while a small portion on the other side is suppressed in development. On this side at maturity the edges of the remainder of the indusium curve in to form a pore.

The receptacle, at first only slightly raised above the surface of the leaf (Fig. 1),

becomes pushed out on the side at first uncovered by the indusium (Fig. 2) and may remain exposed until sporangia appear (Fig. 2). In some sori the indusium covers the receptacle entirely, even before the sporangia are fully defined (Fig. 3).

As the sorus grows older, an elongation of the receptacle goes on (Figs. 3 and 5), the central part of the resulting stalk being continuous with the receptacle, and the peripheral part continuous with the indusium. The side of the indusium in Fig. 4 up almost to the 'kink' represents the portion which later becomes one side of the soral stalk (the portion up to the sharp bend below the start of the indusial flap in Text-fig. 1). The number of cells from side to side across the base of the sorus in Fig. 4 is exactly the same as the number across the stalk of the mature sorus in Text-fig. 1, while the number of cells from *a* to *b* in Fig. 4 corresponds with the number from *a* to *b* in Text-fig. 1. (A counting of the number of cells in length and width of the stalk of the sorus figured in Fig. 13, Ann. of Bot., xxvi, Pl. XXIX, gives precisely the same results. As this sorus was cut from another plant and the figure was made long before the material which forms the subject of this present paper was obtained, it is interesting to observe how closely the sori adhere to one type of construction. Comparison of herbarium specimens collected at wide intervals of time shows how constantly the sori preserve the same size and form.) The elongated condition of the cells of the stalk in Text-fig. 1 suggests what a comparison of Fig. 4 and Text-fig. 1 makes obvious, that, to produce the mature condition, there has been simply a lengthening of the cells at the base of the receptacle, and of the cells of the tissues which in the young sorus are continuous with the indusium. Apparently cell-divisions go on in the superficial layers of the young sorus until sporangia are initiated on the receptacle. Then a lengthening of the individual cells of receptacle and superficial layers produces the stalk of the mature sorus.

The receptacle in the youngest stages examined has in tangential section the outline of an hour-glass. In median section it is shown curved at its tip towards the edge of the leaf (Fig. 1). It widens at its apex as it grows older (Fig. 3); its lower portion becomes constricted, its apex broadly dome-shaped (Figs. 4 and 5). The broad dome-shape is found in the fully developed sorus (Text-fig. 1).

In even the youngest leaves which bear sporangia, the vascular tissue in the veins is defined. The young sorus is always produced below a vein, and the vascular tissue of the vein runs into the base of the sorus. In the earliest stages no tracheidal tissue is present in the upper portion of the receptacle or in the stalk (Figs. 1, 2, and 5), but the cells of the central series, particularly in the lower part of the stalk, become very much elongated, and are narrow even in a fairly young sorus (Fig. 5). When the stalk reaches its full length, the cells of its central region are more or less in process of change into tracheides; tracheides are at the same time defined in the receptacle proper. In the mature sorus the tracheidal system runs as a narrow strip up through the

stalk of the sorus and ends as a fan below the apex of the wide receptacle. Through the base of the stalk this tracheidal system is continuous with the series of tracheides in the vascular strand of the vein upon which the sorus is inserted (cf. *Annals of Botany*, xxvi, Pl. XXIX, Fig. 13).

Sporangia appear upon the receptacle as soon as it begins to lengthen (Fig. 2). The first sporangium occurs in varying positions on the surface—sometimes near the junction of the large indusial flap with the receptacle (Fig. 3); sometimes near the edge of the reduced flap (Fig. 2); sometimes midway between these positions.

The first sporangium is rapidly succeeded by others, which arise close to it. Two sporangia of approximately the same age sometimes occupy positions near the apex of the receptacle, while others very slightly younger appear nearer to its margins (Fig. 4).

A comparison of the condition shown in Fig. 4 with that in Text-fig. 1, in which the oldest sporangia occupy the summit of the receptacle, while young sporangia appear at the margins, leads us to conclude that the sequence is in the main basipetal, but that the sporangia succeed one another rapidly.

In Text-fig. 1 the youngest sporangia figured are at the apex of the receptacle, among the stalks of the oldest. This mixed condition of the mature sorus was figured in *Ann. of Bot.*, xxvi, Pl. XXIX, Figs. 13, 14, and 15. The suggestion made in the earlier paper (*ibid.*, p. 254) that the sorus is of a mixed type upon a Gradate receptacle is confirmed by the details shown in Fig. 4 and Text-fig. 1. The sequence of sporangia shown in these figures proves that the succession of the earliest sporangia is essentially basipetal.

DEVELOPMENT OF THE SPORANGIUM.

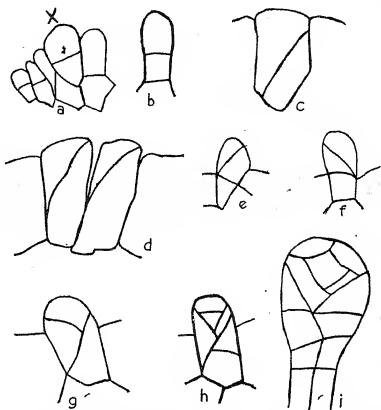
The earliest stages of the development of the sporangium are figured in Text-fig. 2, *a-i*. The cell which becomes a sporangium is often wedge-shaped (*a*). The first wall may be transverse (*a* and *b*) or oblique (*c* and *d*). The oblique wall more frequently occurs, to judge by the condition of various later stages (*g* and *h*). *f* suggests a later condition of the type shown in *b*. Where the first wall is transverse the one which immediately succeeds it is oblique (*e* and *f*), and this second oblique wall commonly meets the first at its junction with the lateral wall (*e* and *f*). Where the first wall is oblique, it may meet the lateral wall at its junction with one of the walls forming the wedge-shaped base of the cell (*g* and *h*); it may meet the lateral wall about half-way down its length (*d*), or it may meet one of the basal walls (*c*). A wall next cuts the oblique wall at right angles (*g*), and that which succeeds this is parallel to the first oblique wall (*h*). In *i* is shown the central cell of the capsule fully formed, while the cells of the stalk are definitely delimited. The later stages in the development of the sporangium and the mature sporangium itself have already been described (*loc. cit.*, p. 254). The mature sporangium is long-stalked, and has an

oblique annulus, the cells of which are continuous past the stalk (loc. cit., Pl. XXIX, Figs. 16 and 19).

We can now consider how these features of the sorus and sporangium affect the systematic position already assigned (loc. cit., pp. 264-5) to *Peranema*.

The character of the vascular system and the structure of the mature sorus and sporangium led to the conclusion that *Peranema* occupied a position intermediate between the Cyatheaceae and the Aspidieae group of Polypodiaceae. A basal indusium of cup-type is characteristic of the genus *Cyathea* and other advanced types of the Cyatheaceae (Bower, '99, p. 52). In *Peranema* the cup is 'developed unequally on its two sides and contracted at its rim, which is turned inwards' (Davie, '12, p. 253). The early stages show that it is of the *Cyathea* type, with a part of one side of the cup lagging behind the rest of the indusium (cf. above, pp. 101, 102).

The stalk is apparently a late growth, due to an elongation of certain cells of the receptacle and superficial layers. The sorus with its basal cup-indusium is certainly of Cyatheaceous type. The form of the receptacle—widely dome-shaped at maturity—recalls that found among the Gradatae (Bower, Land Flora, p. 635). The first sporangia arise commonly at the apex of the receptacle; the subsequent sporangia show clear indications of following in basipetal sequence. But the mature sorus is a mixed one. This shows a step in advance of the Cyatheaceae. The sporangium develops in the manner of one of the Gradatae (loc. cit., p. 638). The wedge-shaped



TEXT-FIG. 2. *a*. A young sporangium, showing a wedge-shaped base. The first division-wall is transverse. $\times 450$. *b*. Another form of young sporangium, with a transverse first division-wall. $\times 450$. *c*. A young sporangium, with wedge-shaped base and oblique first division-wall. $\times 660$. *d*. Two young sporangia, with oblique first division-walls meeting the lateral walls of the sporangia. $\times 660$. *e*. A young sporangium, of the type shown in *a*, with the second division-wall oblique and meeting the first transverse division-wall at its junction with one of the lateral walls. $\times 450$. *f*. A young sporangium of the type shown in *b*, with an oblique division-wall succeeding the first transverse division-wall. $\times 450$. *g*. A young sporangium, of the type shown in *d*, with an oblique second division-wall meeting the first division-wall. $\times 450$. *h*. An older sporangium, of the type shown in *g*, with the covering cell of the capsule defined and an oblique third division-wall meeting the second wall and parallel to the first. $\times 450$. *i*. A sporangium with the stalk and the central cell defined. $\times 450$.

base and the oblique first wall correspond to similar features in the sporangia of *Thyrsopteris* and *Alsophila* (loc. cit., pp. 590, 603). The early segmentations in the sporangia in Text-fig. 2, *e*, *g*, and *h*, are of the Cyatheaceous type. Those found in *c* and *d* are apparently also to be found in some species of *Nephrodium* (Kündig, '88). The early divisions shown in *b* and *f* resemble those in the Polypodiaceous sporangium, to which the mature sporangium of *Peranema*, with its long stalk and almost vertical annulus, bears some resemblance. Thus some sporangia figured resemble those of the Cyatheaceae, others those of the Polypodiaceae. And as a whole the sporangium of *Peranema* is intermediate between those characteristic of the Cyatheaceae and those found in the Polypodiaceae, although there is in it a preponderance of Cyatheaceous characters. We have then to set against a distinctly Nephrodioid vascular system, mixed sorus and 'aspidioid' spores (Davie, '12, pp. 255, 264), a Cyatheaceous indusium and early sporangial segmentation, a Gradate receptacle and early sporangial sequence and spore-number (loc. cit., p. 254). We conclude that *Peranema* is a Fern descended from a Cyatheaceous line and somewhat advanced on the main Cyatheaceous type towards a 'Nephrodioid' type.

This is interesting, apart from general phyletic considerations, in the light which it throws upon the sorus of *Nephrodium*. If the whole of one side of the cup of the Cyatheaceous sorus were to be suppressed, and the other side much developed so as to overarch the receptacle, we should have the type of *Nephrodium*. In *Peranema* we have in the early sorus a partial suppression of the one side of the cup and a great overarching development of the remainder of the indusium. If we for the moment neglect the presence of the stalk, we have in the early *Peranema* sorus the intermediate stage between the type of the Cyatheaceous sorus and that of *Nephrodium*. The extension of the sorus of *Peranema* beyond the level of the leaf-surface seems to permit of an increase of the receptacular area upon which the sporangia may be developed. In well-developed sori the sporangia are actually produced all round the stalk (cf. Pl. III, Fig. 6). Even in the ordinary sorus there is a tendency to extend the receptacle upwards towards the leaf-surface (the figures of the sorus are reversed from their natural position, since in nature the sorus grows vertically downwards from the under surface of the leaf), while the indusium becomes very much stretched out over this extension. The section figured in Fig. 6 was cut transversely to the stalk, just above the junction-point of stalk and sorus, and it shows how the large 'flap' of the indusium becomes extended on the side of the stalk from which it arose and pushed some distance along the stalk as two narrow pouches. In the sori of *Nephrodium* and *Polystichum* the receptacle is present round the stalk and, in *Nephrodium*, forms two pouches on one side of it—making the lobes of the 'kidney'. In *Peranema*, however, the receptacle is always above the indusium; in *Nephrodium* and *Polystichum* it comes to be below.

Apparently there arose in the sorus of *Peranema* a need for space in which to develop more sporangia. Instead of the receptacle being length-

ened from the surface of the leaf, to which the indusium remained attached, receptacle and indusium were shot out beyond the surface, well below which the further elaboration took place. There such extensions and involutions as are figured for one sorus in Pl. III, Fig. 6 had ample room to develop. To conclude the comparison, this parallel between the condition in a mature sorus of *Peranema* and that in a mature sorus of *Nephrodium* helps to strengthen the view that *Peranema* occupies an intermediate position between the Cyatheaceae and the Aspidieae group of Polypodiaceae (which includes the genus *Nephrodium*).

THE PROTHALLUS.

Schlumberger ('11) has described the prothalli of *Diacalpe aspidioides*, Bl., *Woodsia obtusa*, Torrey, and *W. ilvensis*, Br. On the prothallus of *Diacalpe* he found multicellular hairs, mainly on the region of the cushion. These sometimes had glandular heads, less frequently were devoid of them. Similar hairs were found on the prothalli of *Woodsia obtusa*, while on the prothallus of *Woodsia ilvensis* glandular hairs, usually on a one- or two-celled stalk, were present.

Multicellular hairs are present on the prothalli of the Cyatheaceae (Heim, '96). Thus the prothallus of *Diacalpe* resembles that characteristic of that family, while the *Woodsia* prothallus shows a movement from such an affinity towards the Polypodiaceae.

On the prothallus of *Peranema* glandular hairs are present, both on the margins of the wings and on the cushion. Those on the cushion are larger than those on the wings; both types are unicellular, and exactly resemble the terminal cells of the hairs figured by Schlumberger for the prothalli of *Diacalpe* and *Woodsia*. Many of the hairs on the cushion are placed upon slightly raised superficial cells (Pl. III, Fig. 7) and resemble the example figured by Schlumberger from *Woodsia ilvensis* (loc. cit., Fig. 1, 9). At the same time there are many which closely agree with the glandular hairs which are present on the prothalli of *Nephrodium Filix-mas* (Kny, '95, Taf. XCVII and C).

From the structure of the antheridia on the prothalli described by him, Schlumberger was able to form a series linking the Cyatheaceae with the Polypodiaceae (cf. Bauke, '76, and Goebel, '13). *Diacalpe* has a divided lid-cell in its antheridium; *Woodsia obtusa* has a divided lid-cell of two unequally-sized parts; *Woodsia ilvensis* has an undivided lid-cell. In the last species the rupture of the antheridium is brought about by the discharge of the lid-cell, just as in the other two.

Heim ('96) distinguished between the more primitive families of Cyatheaceae, Hymenophyllaceae, &c., and the Polypodiaceae on the method of rupture of the antheridium. In the former the lid-cell was discharged; in the latter it was broken through (loc. cit., pp. 356, 369).

Schlumberger points out that in *Woodsia ilvensis* the remains of the

cuticle which covers the lid-cell persist as a fringe round the neck of the antheridium after the lid-cell has been *discharged*. The resemblance to the ruptured antheridium of the Polypodiaceae is then so close as to destroy the value of the antheridial *dehiscence-method* as a differential criterion (Schlumberger, '11, p. 396). In *Peranema* the lid-cell of the antheridium is generally undivided and is discharged at the maturity of the antheridium, which then presents exactly the appearance of the antheridium of *Woodsia ilvensis*, figured by Schlumberger (loc. cit., Figs. 7 and 8). Only one antheridium was found with a divided lid-cell (Pl. III, Fig. 8), though many were examined in the youngest, semi-mature, and mature stages. This feature of one antheridium, taken along with the features of some of the glandular hairs, mentioned above, shows that *Peranema* retains a suggestion of the Cyatheaceous type in its prothallus, which on the whole approaches even more closely than that of *Woodsia ilvensis* to the type of the Polypodiaceae.

Among the three closely related genera within the Woodsiae-Woodsinae group, to which *Peranema* undoubtedly belongs, the sequence now appears to be *Woodsia*—*Diacalpe*—*Peranema*. *Woodsia* has a Gradate sorus, and prothallial features which relate it to the Cyatheaceae; *Diacalpe* has a mixed sorus, with a basal indusium, and an unmistakable Cyatheoid prothallus, which give it an intermediate position; *Peranema* has a mixed sorus, with a modified basal indusium and a Nephroidoid prothallus, which place it near to the Aspidieae. Already in the features of the sori and prothalli of *Woodsia* and *Diacalpe* we have had indications of a line pointing towards the Polypodiaceae, but clearly originating in the Cyatheaceae. The extraordinary similarity of the vascular systems of *Peranema*, *Diacalpe*, and *Nephrodium Filix-mas* has laid the end of that line in the Aspidieae; the details in the description of the mature sporangia of *Diacalpe* and *Peranema* have confirmed the theory founded on the vascular system (Davie, '12, p. 264). And now in the account of the development of the sorus and sporangium of *Peranema* we have evidence which strengthens this view.

Probably *Peranema* is not itself a link between the Cyatheaceae and the Polypodiaceae; but features in its development, taken in conjunction with features of *Woodsia* and *Diacalpe*, suggest that the process of evolution has moved through types like the Cyatheaceae to types like those found in the Aspidieae group of Polypodiaceae. The account given above of the sorus, sporangium, and prothallus of *Peranema* permits us with confidence to assert that the phyletic line already traced by Professor Bower from the Gleicheniaceae through the Cyatheaceae (Bower, '12) has proceeded through the Woodsiae-Woodsinae group to the Aspidieae group of the Polypodiaceae. Further stages along this line probably moved from *Nephrodium* (of whose sorus we now possess an interpretation) through *Aspidium* and *Polystichum* to certain types included in the comprehensive genus *Polypodium*.

SUMMARY.

1. The sorus of *Peranema cyatheoides*, D. Don, arises superficially on the under side of the leaf; it has a basal indusium, of cup-type, with one portion of the cup suppressed in development, and the other over-arching the receptacle and becoming contracted at its rim; the receptacle is of the Gradate type; the central part of the stalk of the sorus is continuous with the receptacle, and the peripheral part is continuous with the indusium.

2. The early sporangia arise in basipetal sequence; the mature sorus is a mixed one.

3. The sporangium in its early segmentation sometimes follows the Cyatheaceous type, sometimes the type of the Polypodiaceae.

4. The prothallus bears glandular hairs, sometimes slightly raised above the level of the surface upon unicellular bases; the antheridium has been found in every case but one to have an undivided lid-cell.

5. On a general comparison of the features of *Peranema* and the closely related genera in the Woodsiae-Woodsiinae group of Polypodiaceae, a grouping *Woodsia*—*Diacalpe*—*Peranema* is suggested. *Woodsia* comes nearest to the Cyatheaceae, *Peranema* to the Polypodiaceae.

6. The mature sorus of *Peranema* is held to be related to that of *Nephrodium*, and a phyletic line is traced from the Cyatheaceae to the Aspidieae group of Polypodiaceae.

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DESCRIPTION OF PLATE III.

Illustrating Mr. Davie's paper on *Peranema cyatheoides*, D. Don.

Fig. 1 has been drawn with the aid of an Abbé camera lucida.

Figs. 2, 3, 4, 5, 6, 7, 8 have been drawn with the aid of a Leitz drawing apparatus.

Fig. 1. Vertical section through a very young sorus of *Peranema cyatheoides*, D. Don, showing one large indusial flap almost completely covering the receptacle. No sporangia have been developed on the receptacle. At the right side there are one or two cells with thickened cell-walls, representing the laggard portion of the indusium. $\times 225$.

Fig. 2. Vertical section through a slightly older sorus. The receptacle has become extended through the gap between the portions of the indusium. The laggard portion of the indusium is now distinct, and the whole indusium shows a cup-shape. One sporangium is initiated near the lower margin of the receptacle. $\times 150$.

Fig. 3. Vertical section through a young sorus, showing the modified cup-shape of the indusium. One portion of the indusium completely overlaps the receptacle. A sporangium is just initiated near the junction of the receptacle and the large indusial flap. $\times 150$.

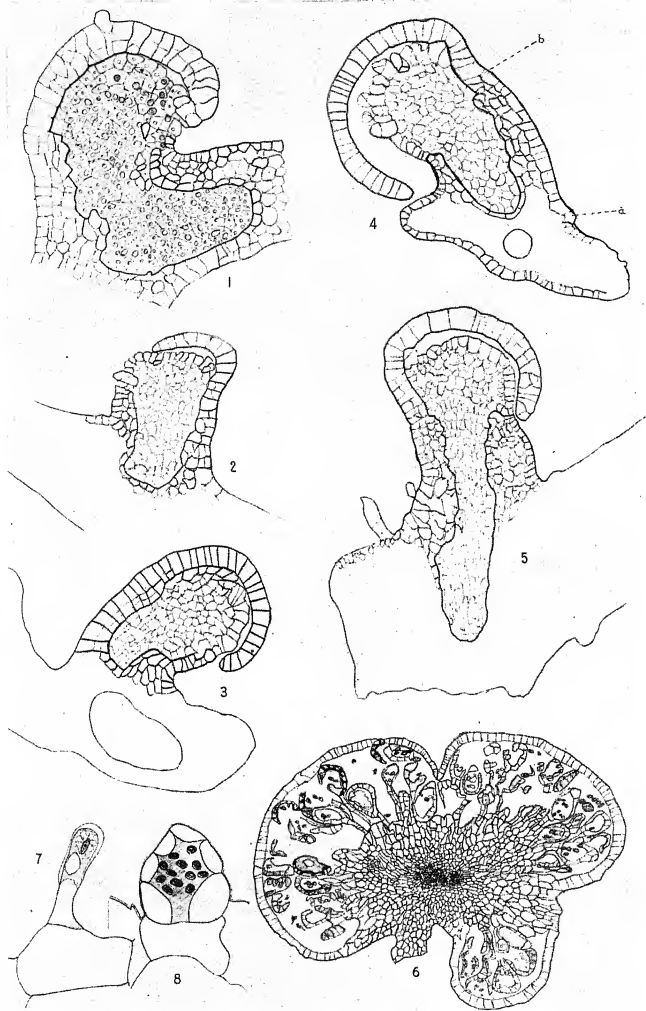
Fig. 4. Vertical section through an older sorus, showing the extension of the large indusial flap over the receptacle, which is now widely dome-shaped. Two sporangia of approximately equal age occupy the summit of the receptacle; younger sporangia occur nearer its edges. $\times 150$.

Fig. 5. Vertical section through an older sorus, showing the extension of the receptacle and the cells continuous with those of the indusium, forming the start of the 'stalk' of the sorus. The cells at the base of the receptacle, in the developing stalk, are considerably elongated and narrow, but no tracheids are at this stage present in the sorus. One sporangium is seen at the summit of the receptacle. $\times 150$.

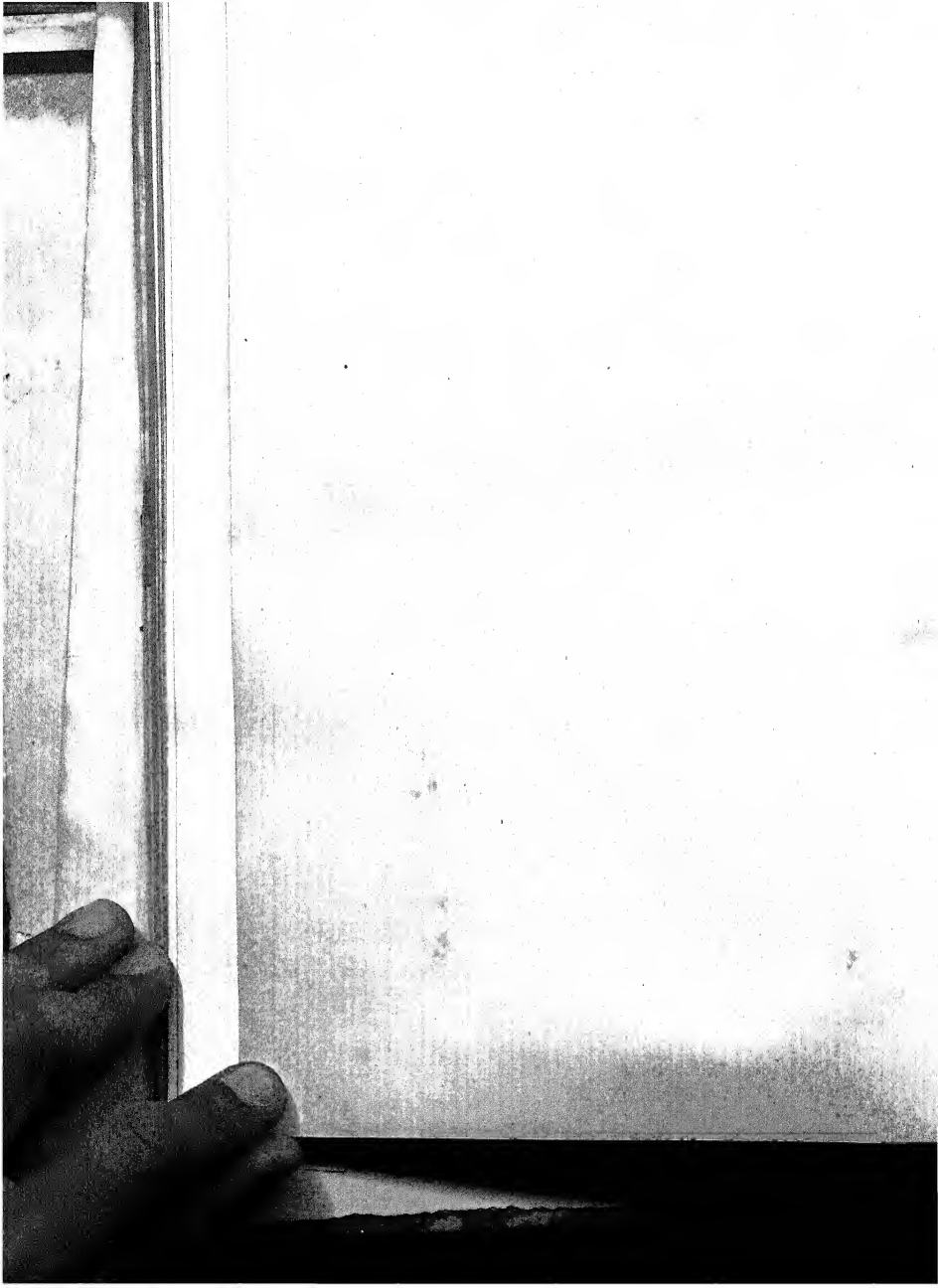
Fig. 6. Transverse section across a mature sorus just above the junction of the stalk and the sporangium-bearing portion. The lips of the large indusial flap are shown on the upper side of the figure. On the lower side, in the right-hand corner is one of the pocket-extensions of the indusium, covering several sporangia. In the left-hand corner is the upper part of a similar pocket, which extends further down the stalk than the right-hand pocket. $\times 35$.

Fig. 7. Unicellular glandular hair from the cushion of the prothallus. It is situated upon a single superficial cell, the wall of which is raised slightly above the general level of the surface of the prothallus. $\times 330$.

Fig. 8. Vertical section through an antheridium, showing a divided lid-cell, and containing several coiled spermatozooids. $\times 330$.



DAVIE — PERANEMA CYATHEOIDES, D. DON.



An Early Type of the Abietineae (?) from the Cretaceous of New Zealand.

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With Plate IV and seven Figures in the Text.

SO little is known of the fossil plants of the Southern Hemisphere in general, and of New Zealand in particular, that the well-petrified tree-trunk from the Cretaceous of New Zealand in the British Museum offered an attractive subject for investigation. Its remarkable structure, which will be described in detail, consists of a striking mixture of Abietinean and Araucarian characters most unexpected in this locality, and affords an interesting addition to our knowledge of the structure and distribution of extinct Conifers.

Last year I described an *Araucarioxylon* from the Cretaceous of Amuri Bluff, New Zealand, which had been sent me by Mr. J. Allan Thomson, Palaeontologist to the New Zealand Geological Survey (see Stopes, 1914). This prepared me to recognize the possibilities of the larger and similarly petrified trunk which I came across in the course of my work in the Geological Department of the British Museum (Natural History). It had the distinctive, black, largely silicified core surrounded by an outer zone of carbonates which was so noticeable in the *Araucarioxylon*. It was with much interest therefore that I learned from the Museum Register that the block had been sent to the Museum by the well-known Australasian geologist Dr. Hector in 1875, and that it also came from Amuri Bluff. Dr. Smith Woodward, F.R.S., kindly gave me permission to have the block cut and to describe it, and I am much indebted to him for his gracious and friendly provision of facilities for examining and studying the new specimen and the others in the Museum required for comparison.

The specimen proved to be well preserved, and its anatomical features are of such peculiarity in their mixture of characters that it is necessary to found a new genus to contain it.

The trunk is large, and is 150 years old or more; the features it shows therefore are those of the mature wood, a point on which I lay stress,

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because much comparative work on fossil wood is vitiated by ignorance concerning the age of the part described, or by comparisons of small twigs of fossils with trunks of living trees and *vice versa*.

The growth rings are strongly marked and regular, thus supporting the geological deduction based on the single specimen *Araucarioxylon Novae Zeelandii*, Stopes, from the same district, that the seasons were well marked in the Upper (or Middle) Cretaceous¹ in the region now New Zealand (see Stopes, 1914).

Before one can discuss the botanical significance of its features, and compare the new specimen with other fossils, it must be described in some detail.

DESCRIPTION OF THE SPECIMEN.

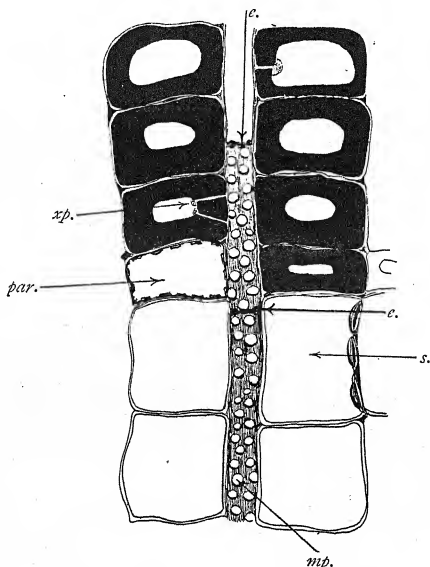
General. The type and only specimen is a thick slab from a trunk not less than 30 cm. x 20 cm. in diameter, and probably more. The position of the pith is apparent, but five chalcedonized cracks centre upon it, so it is not clearly preserved. The greater part of the trunk is petrified in a close-textured, black matrix, largely silica; the hard core in this case measuring 17 cm. across. Round this are outer zones of less well-preserved woody tissue in bands of silicified and carbonate matrix mixed, the proportion of carbonate increasing towards the exterior. The appearance and peculiar mode of petrification is quite similar to the first fossil from this district which I described (see Pl. XX, Fig. 1, Stopes, 1914). None of the outer tissues or bark appears to be preserved.

Topography of the stem. The *pith* appears to be about 1 mm. in diameter, but is too broken for its details, or the details of the primary wood, to be described.

The secondary wood shows very well marked growth rings which are clearly visible to the naked eye in the surface of the trunk which has been cut right across and partly polished. At least 130 rings can be counted in the polished surface, and as the microscopic sections reveal a larger number of rings per radial centimetre than are always apparent to the eye, it is likely that the tree was at least 150 years old, if not considerably more. The growth rings average 0.5 to 2 mm. in thickness, and there is a considerable proportion of thickened late wood in each growth ring (as can be seen in Pl. IV, Fig. 1), which in most cases amounts to half or more than half of the wood of each ring. The rings consist on an average of about ten to thirty tracheides in each radial sequence. In a few places, lying in the radial series between the first spring tracheide and the last late-wood tracheide, are parenchyma cells with thickened and pitted walls. Resin canals and scattered parenchyma are absent.

¹ The exact geological horizon cannot be determined by these two specimens, so for the horizon determination I depend on local geologists.

Medullary rays in transverse section are numerous and conspicuous (see Pl. IV, Fig. 1), and chiefly 1-5 tracheides distant. They are nearly all *uniseriate*, with a few exceptions where the rays are partly *biseriate*. They vary from 1 to 24 cells in vertical height. In 100 counts the most frequent heights were: 3 cells high, 11 per cent.; 4 cells high, 14 per cent.;

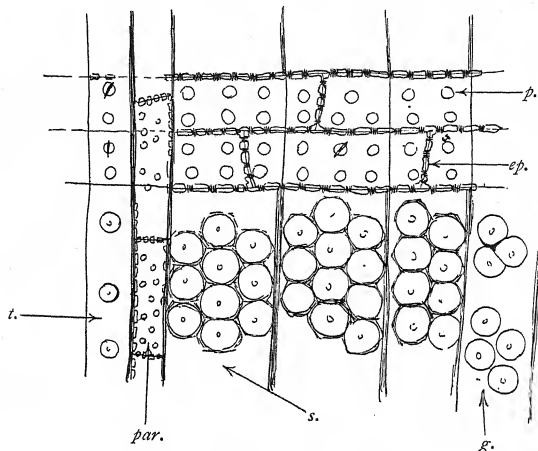


TEXT-FIG. 1. *Planoxylon Hectori*, sp. nov. Small portion of transverse section. *s.*, spring elements in which three rows of pits in the radial wall can be seen. *xp.*, pits in the greatly thickened late-formed wood. *par.*, parenchyma cell between spring and last-formed elements. *mp.*, pits in the horizontal wall of medullary ray cell. *e.*, end walls of medullary ray cells. [Slide No. 52823 a, British Museum (Nat. Hist.).]

7 cells high, 14 per cent.; 9 cells high, 10 per cent.; while rays above 16 cells high amounted to 3 per cent. only. The rays therefore are low for so old a trunk. The cells of the ray are all of one kind.

Details of elements. Tracheides. The large, square elements of the spring wood average about 40×50 to $50 \times 55 \mu$ in diameter. They have rather thin walls (see Pl. IV, Fig. 1, and Text-fig. 1). In their radial walls the three rows of pits are often seen in transverse section (*s.*, Text-fig. 1).

The walls of the later-formed wood elements are greatly thickened, and in them pitting can be seen in the tangential direction in a number of places. The pitting of the spring tracheides is essentially Araucarian, the larger elements having three rows of adjacent alternating pits with hexagonally compressed borders (see Text-fig. 2, *s.*, and Pl. IV, Fig. 2). In several places, towards the ends of tracheides, and in the later-formed elements of each



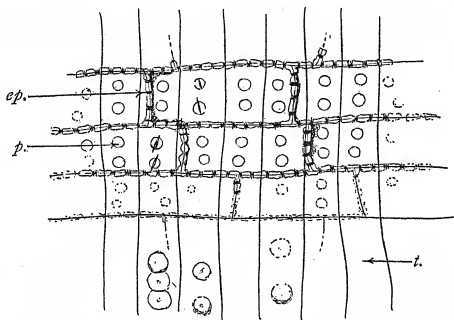
TEXT-FIG. 2. *Planoxylon Hectori*, sp. nov. Radial section through medullary ray at junction of spring tracheides and last-formed elements of previous season. *s.*, tracheides showing the three rows of adjacent hexagonally compressed pits of Araucarian type. *g.*, tracheide with pits in irregular groups. *ep.*, 'Abietinean pitting' of end walls of medullary ray cells. *p.*, pits connecting radial walls of medullary ray cells with adjacent tracheides; note that in the region of the large spring elements these are in three vertical pairs per tracheide-field. *par.*, parenchyma cells lying between spring wood and last element of previous season *t.*, these parenchyma cells are thickened and pitted in all directions: cf. *par.* Text-fig. 1. [Slide No. 52823 c, Brit. Mus. (Nat. Hist.).]

zone, the pits tend to be rounded off and to group themselves irregularly in groups of 3, 4, or 5, very much like those figured in the two left-hand tracheides of Gothan's Fig. 14B, p. 26 (see Gothan, 1907), for his *Cedroxylon transiens*; see Text-fig. 2, tracheide *g.*, in the present paper for comparison with Gothan's specimen.

The later-formed wood has two rows, or one row of adjacent pits, while the latest formed wood has a single row of isolated pits. The average diameter of these bordered pits is 17–18 μ , which appears to be exceptionally large. In spite of what has been written on the subject, I do not feel

satisfied that one can make reliable deductions regarding affinities, &c., from this detail.

The wood *parenchyma* is very inconspicuous in transverse section, though in a few places it can be recognized. The number of elements is not large, and it appears to occur only between the spring elements and the latest formed wood of the preceding season (*par.*, Text-figs. 1 and 2). The cells have nearly straight end walls, and all their walls are thickened and pitted with circular or oval pits. They are seen in Text-fig. 2, *par.*, in radial section, but they are more conspicuous in the portions of the tangential section which happen to pass through the zone between the spring and the last-formed

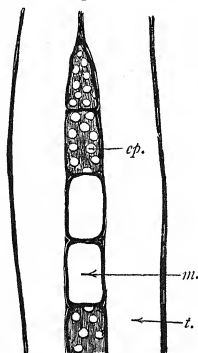


TEXT-FIG. 3. *Planoxylon Hectori*, sp. nov. Radial section through the zone of later-formed wood, showing the characteristic 'Abietinean pitting' of the ray cells and the pitting in the tracheide-fields. *ep.*, pitting of end cells of the ray. *p.*, pits connecting radial wall of ray and adjacent tracheides, note that these are in vertical pairs per tracheide-field, and in some cases distinctly bordered. *t.*, tracheides; cf. Pl. IV, Figs. 3 and 5. [Slide No. 52823 c, Brit. Mus. (Nat. Hist.).]

wood of the previous season. In this view they are numerous, and greatly resemble Gothan's Text-fig. 15, p. 29 (1907) for his *Cedroxylon transiens*.

The *medullary ray cells* appear to be all of one kind, though the end cells of the ray are sometimes slightly more irregular in outline than the others. All the cells have definitely thickened and pitted walls, and these are of the typical 'Abietinean' type. In transverse section the medullary ray cells are seen to tally with two or three of the tracheides, and to have straight end cells. In a number of cases their top or bottom walls can be seen in surface view, when the numerous round pits in them are very apparent (see *mp.*, Text-fig. 1). In radial section the pitting of all six walls can sometimes be seen (see Text-figs. 2 and 3, and Pl. IV, Figs. 3 and 5). The nearly straight or curved end walls and their 'Abietinean' pitting are seen at *ep.* in all three figures. The number of pits per tracheide-field varies

according to the region of the growth zone the elements cross. Where the largest spring tracheides are crossed there are one or two vertical rows of three pits per tracheide-field; while in the later-formed wood there is generally a single vertical pair (see *p.*, Text-fig. 3, and Pl. IV, Fig. 3, and contrast these with *p.*, Text-fig. 2). In some places these pits are clearly bordered, as is shown both in the photograph and the text-figures; in others they have the appearance of being simple pits. The 'Abietinean pitting' of the end walls can often be very clearly seen in tangential sections (see *ep.*, Text-fig. 4, and Pl. IV, Fig. 4).



TEXT-FIG. 4. *Planoxylon Hectori*, sp. nov. Tangential section showing the end walls of the medullary rays and their pitting, *ep.* *m.*, the cells of the ray cut across. *t.*, tracheide. [Slide No. 52823 α , Brit. Mus. (Nat. Hist.).]

COMPARISON WITH OTHER FOSSILS.

No living family has the mixture of characters described, and I am aware of no previously described fossil with which this fossil is identical.

The form described by Gothan as *Cedroxylon transiens* (see Gothan, 1907 and 1910) is perhaps the nearest to it among those previously recorded. Reference to this has already been made on pp. 114, 115. In many respects also the new fossil resembles his *Protocedroxylon araucarioides* (Gothan, 1910). Both these fossils have 'Abietinean pitting' in the medullary ray cell walls, and both have alternating pits in the tracheide walls in the spring wood, which Gothan describes as being quite Araucarian in character. Further comparison with and reference to these two fossils will follow.

In the several American forms described as showing a mixture of Araucarian and Abietinean pitting, the 'Araucarian' features are so often only represented in the tracheide-

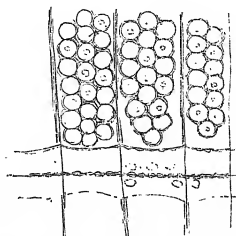
pittings, and there is so subtle a form as to satisfy only the describers of the feature. These tracheide-pits are in single rows, often even isolated, and are often quite round pits which have nothing to distinguish them from ordinary Abietinean tracheides save the reported absence of 'Sanio's rims', a now exploded criterion. With our species it is remotely possible that Jeffrey's *Araucariopitys americana* is to some degree allied (see Jeffrey, 1907), since he describes it as possessing a mixture of Araucarian and Abietinean characters. The tracheides of his plant, however, have round, bordered pits in a single row, and the wood has numerous resin canals; and, further, as his specimens are twigs they can scarcely be compared reliably with our large New Zealand trunk, in any case.

A form which appears, however, to be very closely allied to my new fossil is that which was described long ago by Witham as *Peuce Lindleii* (see Witham, 1833); though the essential characters of the medullary rays of this species were not noticed either by him or by the later writers who mention it.

As I have come to the conclusion that this fossil is sufficiently like the New Zealand form to be included in the same new genus, some notes on its structure and figures of its details not hitherto illustrated will now be given.

NOTES ON *PEUCE LINDELI*, WITHAM (*ARAUCARIOXYLON LINDELI*
OF SEWARD).

This classical species was first described from a specimen of Upper Lias age found one mile south of Whitby by Mr. Nicol. In 1831 Witham partly described and figured it, but without giving it a name. In 1833 he amplified his description and added further plates to those he reprinted from his earlier work, and named the fossil *Peuce Lindleii* in the text, and *Peuce Lindleyana* in the plate description. He paid particular attention to the specimen, as it was the first which had been discovered at that time which showed what appeared to him to be direct affinity with living Conifers. Unger, Brongniart, Goepfert, Carruthers, and others have referred to it under various names from time to time, and it was re-described and figured in more detail by Seward in his Jurassic Flora (see Seward, 1904, p. 56 *et seq.*). Seward calls it *Araucarioxylon Lindleii*, basing his generic identification on the tracheide-pitting, which he describes as follows: 'Tracheids bearing 1-3 rows of bordered pits on their radial walls; the pits usually occur in two contiguous rows, alternately disposed and polygonal in shape; occasionally three rows of pits occur, and some of the tracheids possess a single row.' He figures in his Pl. VII, Fig. 5, their typical Araucarian pitting (see also my Text-fig. 5).

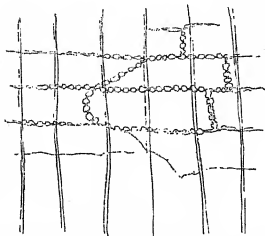


TEXT-FIG. 5. *Planoxylon Lindleii* (Witham). Sketch of radial section showing the Araucarian type of pitting of the tracheides and something of the 'Abietinean pitting' of the ray cells of a medullary ray two cells high. [From No. 51488, British Museum (Nat. Hist.), Geological Collections.]

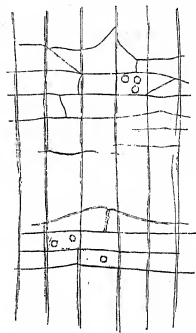
The original old sections and some others are now in the Geological Department, British Museum (Natural History), and I have recently examined them carefully, and have observed that not only do the general characters of the tracheides and their pittings agree with the New Zealand fossil, but that the much more remarkable features of the ray cells also agree very closely. In the sections now in the British Museum, particularly

in slides 51488 and 51724, the thickening and 'Abietinean pitting' of a number of the medullary ray cells can be clearly seen. Some sketches from these are given in my Text-figs. 5, 6, and 7.

At the time Seward wrote (1904) the diagnostic importance of the medullary ray cells was not fully realized, and he gives no figures of the details of their walls. Regarding the rays, he says: 'Medullary rays distinct in transverse section of the wood, composed of narrow, radially elongated cells, with single pits in their walls; the rays vary in depth from a single row to more than twelve rows of parenchymatous cells.' Further on he says of the rays that 'they consist of radially elongated parenchymatous cells which occasionally exhibit small pits, and the cavities are often



TEXT-FIG. 6. *Planoxylon Lindlei* (Witham). Cells of medullary ray in radial view, showing the thickening and 'Abietinean pitting' of all the walls. [From Slide No. 51488, British Museum (Nat. Hist.).]



TEXT-FIG. 7. *Planoxylon Lindlei* (Witham). Sketch from a part of Section No. 51724, British Museum (Nat. Hist.) Geological Collections, to show the outline of the cells of two medullary rays with the irregular terminal cells suggestive of the Abietinean feature of ray tracheides.

full of vacuolated brown contents.' The radial view of the medullary ray cells given by Witham is merely a series of straight lines. It is therefore necessary now to describe the detailed character of these cells.

In a few cases in transverse sections, I have seen portions of the top and bottom walls in surface view similar to those described in the New Zealand specimen and figured in Text-fig. 1, *mp*. In the tangential sections also, e. g. in slide 51488 (British Museum), faint suggestions of these pittings can be seen. But in a number of places the radial sections retain quite clearly the thickening and pitting of the ray cell-walls. A typical ray is sketched in Text-fig. 6 from slide 51488. Here the characteristic thickening and

pitting of the top, bottom, and end cells can be seen clearly. It is more difficult to determine the exact nature of the pits in the radial walls, but there seem to be rows of about three in the tracheide-field where the rays cross the spring tracheides, and a smaller number in the regions of the later-formed wood. The cells of the edge of the ray have rather irregular outlines (see Text-fig. 7), reminding one of the early stages of ray-tracheide formation, an Abietinean feature.

With these characters, the rays of *Peuce Lindleii* are quite unlike those of any true Araucarian, living or fossil, and are strikingly similar, as are its tracheides, to those of the new fossil. In addition, the distribution of the wood parenchyma is alike in the two forms.

For various reasons, some of which will be dealt with in a later paragraph (see p. 122), a new 'genus' seems to me to be required to contain these two fossils, and possibly some others. Regarding the nature and value of these artificial 'genera' I have recently written at some length (see Stopes, 1915, p. 58 *et seq.*) before the present data came to light. As will be seen on reference to these pages, I take an extremely conservative view, and consider that the fewest possible number of such 'genera' should be used. The foundation of the present genus, however, appears to me to be essentially necessary.

Planoxylon, gen. nov.

[Based on the Greek root *πλανᾶσθαι*, the same as in Planospores; the suggestion being that the forms comprising the 'genus' were moving from one position to another in a systematic sense—we do not know how near any of our present genera this genus-complex was, but presumably, though by no means certainly, it lay between the Araucarineae and the Abietineae.]

Diagnosis. Coniferous wood without, or with occasional, resin canals. Regular growth rings. Tracheides with alternating, hexagonally bordered pits (2 or 3 rows) in spring wood; later-formed wood with single rows of adjacent or isolated pits. Pits present in tangential walls of late-formed wood. Rays almost entirely uniseriate, locally a few may be partly biseriate. Typical 'Abietinean pitting' of ray cells well marked, apparent in transverse, radial, and tangential sections. Radial walls of ray cells pierced by a small number of pits per tracheide-field (1-3 vertical pairs according to position in growth ring), these pits sometimes clearly bordered. Wood parenchyma present between spring and last-formed wood of previous season.

The genus founded on the wood of fair-sized branches and trunks.

In the genus are placed at present two species, viz. *Planoxylon Lindleii* and the new species from New Zealand (see, however, note on p. 120).

1. *Planoxylon Lindlei* (Witham).

1831. [no name] described and figured, Witham, 'Observ. foss. Veg.'

1833. *Peuce Lindlei* (later *P. Lindleyana*), Witham, 'Intern. Struct. foss. Veg.', p. 58 *et seq.*, Pl. IX, Figs. 1-5; Pl. XV, Figs. 1-3.

[Various references as *Pinites* or *Peuce Lindlei* by Unger and others, see Seward, 1904].

1904. *Araucarioxylon Lindlei*, Seward, 'Jurassic Flora', vol. ii, p. 56 *et seq.*, and Pl. VI; Pl. VII, Figs. 2, 3, 5.

Species based on branches, some not less than 12 cm. in diameter.

Diagnosis. Growth rings very distinct. Medullary rays from 1-12 and more cells in vertical height; end cells of ray irregular in outline in radial section, suggestive of early stage of ray-tracheide formation. Pitting, &c., characteristic of genus. Wood parenchyma scanty. Resin canals irregularly present, principally in spring wood.

Horizon: Upper Lias.

Locality: One mile south of Whitby, Yorkshire, England.

Type: Witham's specimen, found before 1831 by Mr. Nicol. Slide No. 51484 British Museum (Nat. Hist.). *Other figured specimens*: Nos. 51488, 51449, 51724, Geol. Depart. British Museum (Nat. Hist.), see Seward's Catalogue, 1904.

2. *Planoxylon Hectori*, sp. nov.

Text-Figs. 1-4, Pl. IV, Figs. 1-5.

Species based on large trunk, over 150 years old.

Diagnosis. Growth rings very distinct. Spring tracheides 40-55 μ in diameter, with three rows of hexagonally bordered, alternating bordered pits, each averaging 17-18 μ in diameter. Intermediate elements with pits in irregular groups, late wood with single rows of isolated pits. Medullary rays 1-21 cells high, chiefly 3-9 cells high. Ray cells all alike. 'Abietinean pitting' very conspicuous. Pits in radial walls of rays in vertical pairs, 1-3 per tracheide-field. Wood parenchyma apparently infrequent, all cells thickened and pitted, walls rectangular. Resin canals apparently entirely absent.

Horizon: Cretaceous (Upper or Middle).

Locality: Amuri Bluff, New Zealand.

Type (and only specimen): No. 52823 British Museum (Nat. Hist.) Geol. Dept., presented in 1875; and six slides cut from it in 1914.

Collector: Probably Dr. Hector.

Note.—I think it is extremely probable that both Gothan's *Cedroxylon transiens* and his *Protocedroxylon araucarioides* should be included in this genus; but I hesitate to transfer them to it because I have not seen his original specimens, and though his figures do not demonstrate all the

characters of the wood, they indicate differences which *may* be important, while Gothan himself accentuates the Abietinean affinity of his two fossils.

Gothan's species *Protocedroxylon araucarioides* is identified by Holden (see Holden, 1913, pp. 538-9) in the Jurassic of Yorkshire, and re-named by her *Metacedroxylon araucarioides*, regardless of the laws of nomenclature, and without diagnosing her new genus. In the same 'genus' she has quite recently (Holden, 1915) included a new species, *M. scoticum*, Holden, though in her description she states that 'the pits of the tracheids are confined to the radial wall, where they are strictly uniseriate', and that in the medullary rays the transverse walls are thick and heavily pitted, and 'radially there are one, or less frequently, two, half-bordered pits to each cross field, similar to those of *Podocarpus*, or certain species of *Pinus*'. As she has not diagnosed her genus, and as her figures of the new species do not illustrate some most important details of the rays, it is impossible to determine how closely her new wood is allied to Gothan's species *Protocedroxylon araucarioides*, and, consequently, to our own new genus.

AFFINITIES.

I have so recently, and at some length, gone into the diagnostic value of various details of wood structure (see Stopes, 1915) that I do not wish now to reopen the extensive discussions involved. In connexion with the two interesting forms now described, it will suffice to recall the fact that there are those who lay chief stress on the tracheide pitting (and this group includes many of the older workers, Lignier, 1907, and at present principally Professor Jeffrey and his school, with their faith in the 'bars of Sanio'); and the other workers who, in the main, lay greater stress on the details of ray structures. While finding no single character infallible, I have observed, both in living and fossil forms, such a remarkable specific stability of character in the details of the ray, that in my opinion Gothan's work in drawing attention to the ray structures (see Gothan, 1905, '07, '10) is invaluable. And I incline (in spite of certain exceptional cases), where the evidence from different details conflicts, to give greater weight to ray-structures as indicators of systematic position than to any other *single* feature.

The presence of such medullary rays as have just been described and figured in the two Mesozoic fossils under consideration, therefore, in my opinion, renders it impossible for the plants to have been either Araucarians in a modern sense, or closely allied to the complex of 'Dadoxylon' forms (Cordaitean-Araucarian), highly suggestive of this affinity though the tracheide-pitting may be. It should be remarked that in the species of *Araucariopitys* and other such forms described as having a mixture of Araucarian and Abietinean features, the 'Araucarian' features of the tracheides are minute and often debatable points in the characters of the *single row of pits*. Now in the new fossil we have much less debatably,

indeed uncompromisingly Araucarian pitting. It is therefore to be expected that the tug-of-war between the two schools of thought will be nearly balanced, as the fossil affords such a typical case of both Abietinean and Araucarian features.

Gothan's forms *Protocedroxylon araucarioides* and *Cedroxylon transiens* with pitting, but slightly less Araucarian than in the new fossil, are included by him in the Abietineae, as he testifies in the names he gives them. Yet, greatly as I value the medullary ray structure as a diagnostic feature, I cannot follow Gothan in this. The position appears to me to be this: In our living flora certain characters have been found to be, on the whole, characteristic of certain types; the alternating hexagonal pitting of the tracheides—of the Araucarineae; the 'Abietinean' thickening and pitting of the rays—of the Abietineae; the ray-tracheides—of the Pinaceae; and so on. But when we get back to the Mesozoic Gymnosperms which undoubtedly include many forms leading from one group to another, many of which are entirely extinct, we cannot be justified in saying, 'where we find a mixture of characters, that one or other of the features makes the wood a representative of one or other of the living families in which it occurs. The fossil may very well belong to a totally extinct family, the existence of which is as yet unsuspected by us. I feel, therefore, that it is unjustifiable to name such specimens (whose fructifications are unknown to us) in a way to indicate definite affinity with any living group. Hence Gothan's use of *Cedroxylon*, *Protocedroxylon*, as well as Holden's use of *Metacedroxylon*, all seem to be illegitimate. That Gothan uses such names is the more surprising since he so clearly sees the problem in the parallel case of the wood of *Voltzia*, regarding which he says (Gothan, 1910, p. 31): '... in den *Voltzia*-Schichten so gut wie nur araucarioid getüpfelte Stämme vorkommen; und es wird wohl kaum jemand *Voltzia* wegen dieser Hoftüpfelung für eine Araucarie erklären; ihre Verwandtschaft ist bei den Taxodien zu suchen.' As is, I should perhaps add for the sake of those unacquainted with the palaeontological evidence, very clearly indicated by the structure of the cone scales of *Voltzia*.

To what group or extinct intermediate group the new *Planoxylon Hectori* belongs, its name does not attempt to indicate. Nevertheless, having said sufficient to indicate the caution which it is necessary to exert, it is worth while playing with the idea that the genus had leanings out towards, if not actual affinities with, the Abietineae. The highly and characteristically developed 'Abietinean pitting' of its rays supports this view by the best known diagnostic character in mature woods. This idea is of particular interest when we remember the *locality* in which the fossil was found, viz. New Zealand.

There are no living Abietineae or Juniperineae in New Zealand or Australia to-day, as reference to the floras of the district at once indicates

(see Bentham, 1873, and Cheeseman, 1906). In New Zealand, indeed, the only endemic Conifers are the genera *Agathis*, *Librocedrus* (a misleading name—the plant has nothing whatever to do with *Cedrus*), *Podocarpus*, *Dacrydium*, and *Phyllocladus*. Not one of these forms has structure at all like the fossil, so far as present discoveries go. The ray structure of the Podocarpaceae, as is well known, is typically very different from that of the Abietineae.

A word of warning must be uttered: this new fossil, with its mixture of Araucarian and Abietinean features, can NOT be taken as evidence of the Abietinean origin of the Araucarineae now living in Australasia. It is millions of years younger than other fossils with true and normal Araucarian characters, from many parts of the world.

It is, however, highly suggestive of the view that an extinct group of unexpectedly Abietinean affinity inhabited parts of the Southern Hemisphere in the Mesozoic epoch.

Palaeobotany is beginning to accustom botanists to the idea that Australasian forms, *Araucaria* itself for instance, inhabited this country and Northern Europe in Mesozoic and Tertiary times. We must now prepare to entertain the reverse idea, that in the past the typically northern types of Conifers were inhabiting New Zealand at about the same time.

Whether or not *Planoxylon Hectori* was directly Abietinean in its affinity, it was much more closely allied in the structure of its wood to living Abietineae than is any form now endemic in Australasia so far as is at present known.

SUMMARY.

1. A petrified tree-trunk of not less than 150 seasons' growth is described from the Cretaceous of Amuri Bluff, New Zealand.

2. Its medullary ray cell-walls are thickened and pitted with typical 'Abietinean' pitting; and it has also wood parenchyma between the spring and last-formed wood of the previous season.

3. Its spring tracheides have three rows of hexagonally compressed adjacent bordered pits; the next formed elements have groups of round-bordered pits; and the last-formed elements have pits in single rows.

4. It is very similar to *Peuce Lindleii* of Witham, the detailed structure of whose rays is now described for the first time.

5. The new wood and Witham's species are placed in a new genus, *Planoxylon*, which is diagnosed on p. 119. It is recognized that it may be an extinct genus of unknown affinity, or may be more closely allied to the Abietineae than can be at present proved.

6. It is probable that Gothan's *Cedroxylon transiens* and *Paracedroxylon araucarioides* should also be included as further species of this genus.

7. The new fossil *Planoxylon Hectori* is particularly interesting as

it comes from Australasia, where no Abietineae or Juniperineae or other forms with 'Abietinean' ray structure are endemic at present.

8. The well-marked growth rings, with their large proportion of greatly thickened elements, confirm the conclusions regarding the climatic conditions of this region at the time the plant was alive, which were drawn from the first specimen (*Araucarioxylon Novae Zeelandii*) described from Amuri Bluff, and held to indicate the contemporary existence of well-marked seasons.

The expenses incurred in photographing the specimens were defrayed out of a Royal Society Grant.

DESCRIPTION OF PLATE IV.

Illustrating Dr. Stopes's paper on *An Early Type of the Abietineae* (?).

FIG. 1. Transverse section of wood, showing the well-marked growth rings and large square spring tracheides.

FIG. 2. Radial longitudinal section of the spring tracheides, showing the typical Araucarian type of pitting. In the rays indications of the rows of three pits per tracheide-field can be seen.

FIG. 3. Radial longitudinal section of a ray, showing the vertical pairs of pits per tracheide-field, *p.*, and the 'Abietinean pitting' in the end wall, *ep.* (cf. Text-fig. 3).

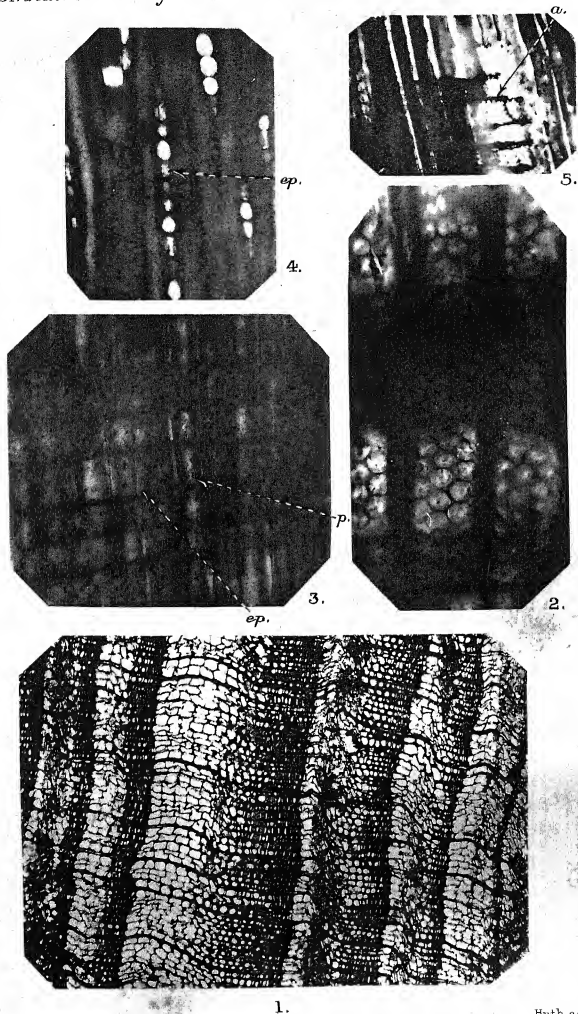
FIG. 4. Tangential longitudinal section of rays, showing the 'Abietinean pitting' of the end wall, *ep.*

FIG. 5. Lower power view of radial longitudinal section, showing at *a* the 'Abietinean' thickening and pitting of the ray-cells.

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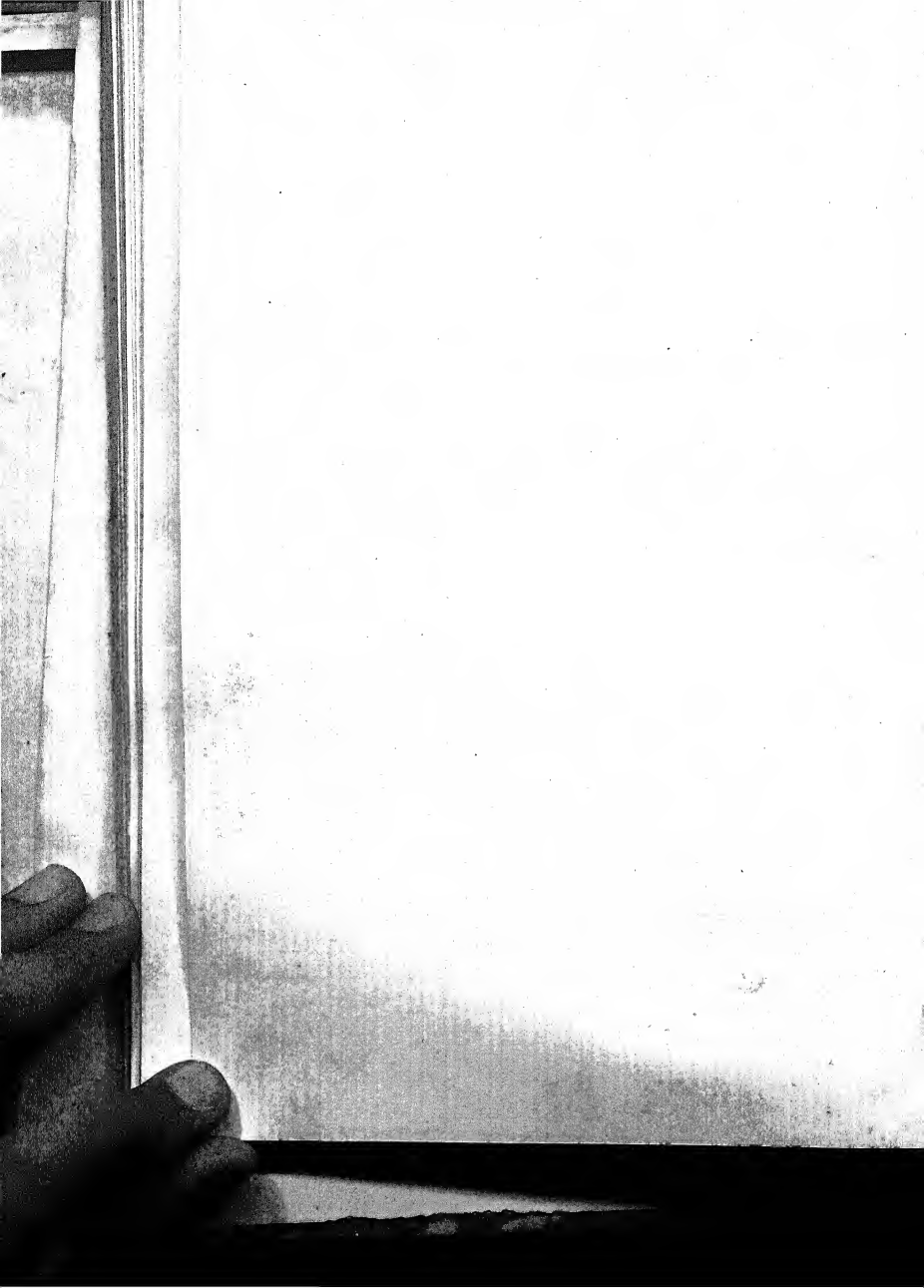
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Huth coll.

STOPES-PLANOXYLON HECTORI, nov. gen. et sp.



Further Observations on the Wound Reactions of the Petioles of *Pteris aquilina*.

BY

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With four Figures in the Text.

IN 1912 I recorded in this journal¹ some observations on the wound responses of filicinean petioles. Whilst in Cumberland during the summer of 1914 the opportunity was taken of collecting material of *Pteris aquilina*, in which specimens showing wound-scars of greater or less extent are not uncommon. This material was supplemented by a further supply collected at Oxtou (Notts.). Specimens such as these suffer, of course, from the disadvantage that one cannot determine either the cause of the injury or the age of the wound, but, on the other hand, they afford some evidence as to how far the wound response exhibited under natural conditions agrees with the results obtained experimentally. The injured petioles may be grouped, for purposes of description, under two heads as follows:

(i) Those in which the wound did not penetrate below the sub-epidermal sclerenchyma. These were very common.

(ii) Those in which the sclerenchyma had been penetrated. The wounds of this second type naturally varied in severity, and in some cases were fairly deep seated.

Whilst differing in points of detail, the whole of the specimens examined showed a certain number of well-marked features in common, namely:

(i) The occurrence of a bright yellow substance in the walls of the zone of cells abutting on the wound. This discoloration, which was due to the deposition of a tannin-like substance,² became more pronounced and darker in tint in the most superficial layers.

¹ Holden, H. S. : Some Wound Reactions in Filicinean Petioles. *Ann. Bot.*, 1912, p. 777.

² It gives a greenish-black coloration with neutral ferric chloride, and a red coloration with an aqueous solution of iodine in KI mixed with a little 10 % ammonia. (Cf. Haas and Hill, *Chemistry of Plant Products*, pp. 190-7.)

(ii) The partial or complete degeneration, in the wound area, of the lignified elements constituting the sub-epidermal armour.

(iii) A more or less pronounced thickening of the cell-walls, sometimes of a purely cellulose nature, but often accompanied by partial or complete lignification.

Taking now the first of the two types of wound referred to above, it is found that the nature of the response is somewhat variable. Where the wound is extremely slight—that is to say, when the wound surface does not penetrate below the fourth or fifth layer of cells—there is very little obvious effect, except for the yellow discoloration alluded to. Microchemical tests demonstrate, however, that in the majority of cases the most superficial cells of the sclerenchyma have become delignified, and give the cellulose reaction with chlor-zinc-iodine.¹ If, on the other hand, the sclerised armour is almost penetrated, there is very generally a compensatory thickening of the cortical elements immediately below the wound surface, such thickening being normally of a cellulose or ligno-cellulose character. In about 20 per cent. of the cases examined, however, lignification appeared to be complete. These differences may of course be due to differences in the age of the wounds, but, judging from appearances, this is unlikely. The walls of the affected cells are abundantly pitted and do not appear in any way degenerate, except possibly for the yellow colour (cf. Fig. 1, v). The effect on the sclerised elements themselves, apart from delignification, is also of some interest, as in many cases a considerable increase in the thickness of the wall accompanies this process. This feature is well illustrated in Fig. 1, iii and iv, in which the walls of the sclerenchyma on the flanks of the wound show a marked increment which is entirely of a cellulose nature and is often obviously stratified. With regard to wounds of the second type, namely, those which penetrate the sub-epidermal sclerenchyma, the reaction is found to be extremely variable. In a large number of cases the plant exhibits merely a somewhat extensive local thickening of the cells in the affected area (Figs. 1, i, ii; 2, i, &c.). The modified cells always extend more deeply into the tissues of the petiole in the neighbourhood of the vascular strands (Figs. 1, ii; 2, ii), and in many cases the latter are wholly or partly flanked by patches of thickened tissue which are quite disconnected from the main mass (Figs. 2, iv; 4, i). As in the less severe types of wound described above, the amount of lignification varies considerably, but the superficial uninjured cells practically always give a cellulose reaction, whilst those below stain pink or red with phloroglucin after acidification with HCl. The cause of the cellulose reaction of these more superficial cortical elements is something of a puzzle, but it may be that their nearness to the wound surface acts deleteriously upon them, and thus prevents their reacting as fully as those which are

¹ See Appendix.

deeper seated. In addition to the reaction to phloroglucin given by the obviously thickened elements, a red coloration frequently occurs in the adjoining cortical tissue in which there appears to be no thickening, so that it is evident that lignification is produced to some extent in these tissues also.

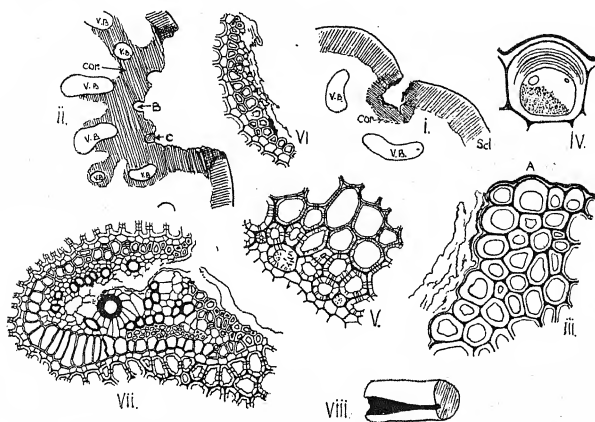


FIG. 1. i. Diagrammatic transverse section of wound area showing compensatory thickening of cortex. ii. Diagrammatic transverse section near middle of wound area shown in i. Note the spread of the thickened tissues in the neighbourhood of the vascular strands. iii. Small portion of i, showing the delignified sclerenchyma on the flank of the wound and its increased thickness. iv. A single cell (A) from iii, showing the stratification of the wall. v. A small portion of the thickened cortex in the vicinity of a bundle. vi. Remains of bundle ii, B, showing the thickened cells of the starch sheath. vii. Remains of bundle ii, C, showing thickening and in some cases elongation of cells of the starch sheath. viii. Portion of wounded petiole from which i and ii were cut, i being from the narrow end, ii from the broad end. i and ii $\times 12$, iii $\times 350$, iv $\times 650$, v $\times 250$, vi and vii $\times 500$, viii $\times 1$. scl. sub-epidermal sclerenchyma; cor. thickened cortical cells.

A second and more pronounced type of reaction which occurs fairly frequently is that of the radial elongation of the cortical cells in the wound area (Fig. 2, iv). This phenomenon is usually accompanied by an increase in the thickness of the cell-walls, as in the cases previously described. Between the two types of reaction, namely, thickening with elongation, and thickening alone, no sharp line of division can be drawn, since in many the more vigorous type of response occurs in the middle of the wound area whilst absent from the two ends. Moreover, there are instances in which a local patch of cortical parenchyma has elongated and become thickened, whilst throughout the rest of the wound no such elongation is manifest (Figs. 3, i; 2, ii, c). Local growth of this character seems in no sense

dependent on the proximity of the vascular strands, and there appears to be no satisfactory explanation of its occurrence.

In some few cases the stimulus of wounding seems to have produced very far-reaching results, and to have caused elongation through quite a large part of the cortex. Such a case is illustrated by Fig. 3, i, iii, iv.

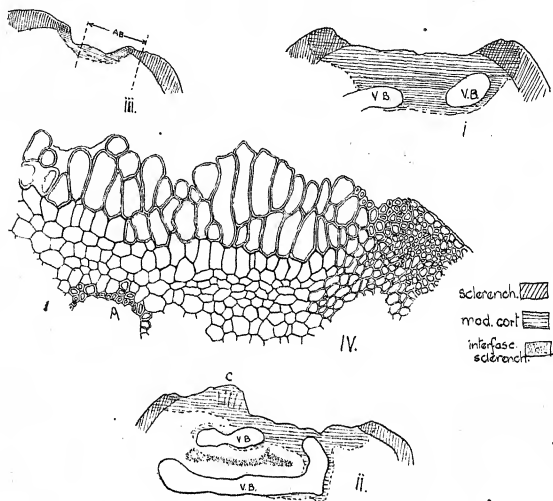


FIG. 2. i. Diagrammatic transverse section of wound area, showing a specimen in which thickening of the cortical cells has taken place to a considerable depth. ii. Diagrammatic transverse section, showing extension of thickened cells in the region of the bundles, and at C, local elongation of the cells. iii. Diagrammatic transverse section in which a relatively shallow cortical zone has been affected. iv. The portion AB of iii, showing elongation of the cells with thickening. Note at A the local thickening in the bundle region. i, ii, and iii $\times 12$; iv $\times 300$.

Here it will be seen that fully half the cortical parenchyma is affected, and that the wholesale elongation shows some very interesting features. The radial extension of the cells seems to have developed what may almost be termed 'lines of flow', some spreading fanwise from the vascular strands, others flowing round these obstructions (Fig. 3, ii). As is to be expected, these lines of flow come into contact in various parts of the petiole, this resulting in their mutual arrestment. Such contact regions are indicated by a separation line of gummy deposit of a yellow or brown colour (Fig. 3, iii and iv). Occasionally contact occurs between several lines of

flow, as in Fig. 3, iv, in which case the gummy deposit appears branched in a triradiate or quadrate manner, as seen in transverse section.

The discussion of the effect of wounding on the vascular bundles has been deferred till last, because it seems to have little or no connexion with

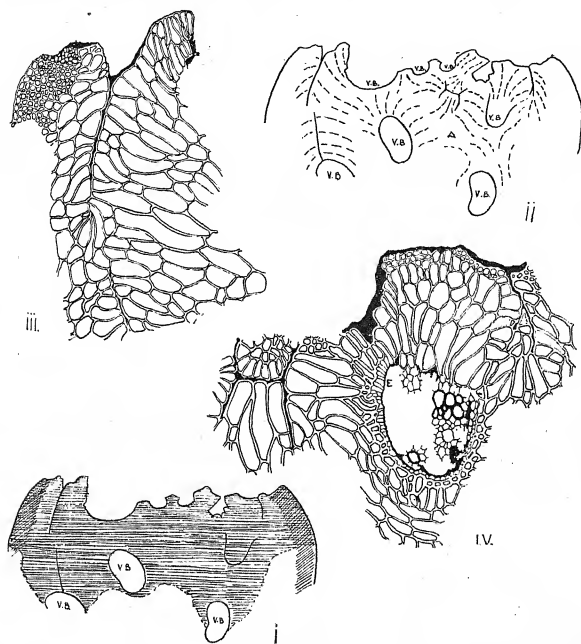


FIG. 3. i. Diagrammatic transverse section of wound area, showing the cortical cells affected to an unusually large extent. ii. Tracing from i, indicating the 'lines of flow' exhibited by the elongated cortical cells and also the lines of contact. iii. Portion of ii, more highly magnified to show detail. This is from the left of ii. iv. Portion from the right of ii. Note the elongation of the endodermal cells at x. i and ii $\times 12$, iii and iv $\times 300$.

the nature of the traumatic response in the general ground-tissue. The tissues¹ comprised in the vascular bundles show no reaction when the wound is purely superficial and does not penetrate the sclerenchyma. Apart from the occasional discoloration of the xylem and phloem elements,

¹ The endodermis is included here.

the deeper wounds also appear normally to have no effect, although, as in the case of the petiole illustrated in Fig. 3, iv, disintegration of the bundle may occur, but this is comparatively rare. In the few instances in which there is an obvious reaction, the vascular bundles affected either abut on or are adjacent to the wound surface. The traumatic response is always confined to the non-specialized constituents of the bundle, such as the starch sheath and the conjunctive parenchyma, the only exception noted during

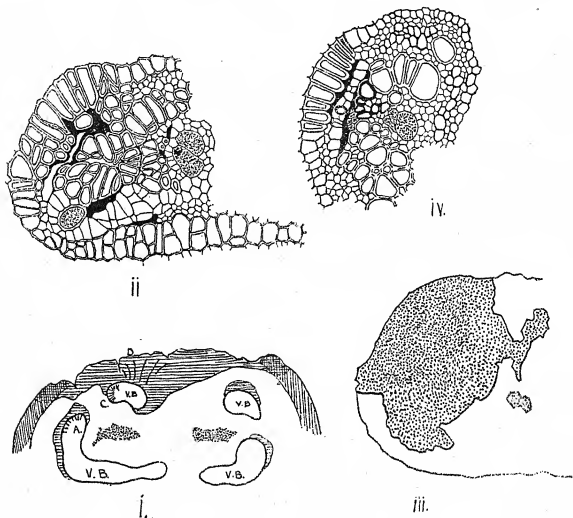


FIG. 4. i. Diagrammatic transverse section of wound area, showing pad of thickened cortical cells at wound surface and also isolated masses of thickened tissue on the flanks of the bundles. Local elongation of the cortical elements occurred at D, and the tissues of the vascular strands were affected at A and C. ii. Affected portion of C, showing effect of wound on starch sheath and conjunctive parenchyma. iii. Tracing from ii. The dotted area represents the cells which were discoloured by tannin. iv. Affected portion of C. i $\times 12$; ii, iii, and iv $\times 500$.

the present observations being that shown in Fig. 3, iv, in which the endodermis had elongated at one point. With regard to the starch sheath, this may simply develop thicker walls, or may become both elongated and thickened. These stages are well illustrated in Fig. 1, vi and vii. The wound in this particular case had caused the total destruction of some of the vascular strands and the partial destruction of two others (Fig. 1, ii, B and C respectively). Of the strand B only a portion of the starch sheath

was left, and this had thickened considerably (Fig. 1, vi), whilst of the strand C about half remained. In this the starch sheath had also thickened, and in addition elongation of the cells had occurred on the side remote from the wound surface (Fig. 1, vii). The remaining xylem and sieve-tubes showed a bright yellow coloration, and there were some indications of elongation in the case of the conjunctive parenchyma. A further illustration is afforded by the vascular bundles shown in Fig. 4, i, ii, and iv. The reaction only affected one end in each of the two bundles and, as in the previous instance, the elements composing the starch sheath had elongated and thickened, but in addition many of the cells had divided by a transverse wall. The conjunctive parenchyma was much more obviously active in the smaller and more superficial strand than in any other instance noted. Many of the cells were thickened and displaced, and in one small portion, in which the cells had remained thin-walled, a small cambiform patch was developed. All the thickened cells were bright yellow with tannin, and gave a cellulose reaction.

One other point remains to be mentioned, namely, the absence of the gum deposit which was so constant a result of the traumatic stimulus in artificially wounded forms.¹ In the present series, gum deposits only occurred in the cavity parenchyma of affected bundles and in occasional tracheides. Apart from this one feature, the examination of the petioles confirms the results arrived at experimentally in the earlier paper with regard to ferns of the type of *Pteris aquilina*.

SUMMARY.

1. Petioles of wild *Pteris aquilina* often show wound-scars. The majority of the wounds are very superficial, not penetrating the sub-epidermal sclerenchyma; others are deeper seated.

2. The wound reactions are somewhat variable, but are characterized by (i) a compensatory local thickening, and partial or complete lignification of the cortical parenchyma, which may or may not be accompanied by elongation, (ii) the local delignification of the sub-epidermal sclerenchyma, (iii) a deposit of tannin in the cell-walls in the affected area.

3. Wound reactions in the tissues composing the vascular strands are rare, and where they do occur are confined to the starch sheath and conjunctive parenchyma, which thicken and may elongate and divide.

4. The results obtained are confirmatory of those produced experimentally.

¹ Holden, loc. cit.

APPENDIX.

The phenomenon of local delignification in the sub-epidermal sclerenchyma is so characteristic and so unexpected a feature of wounds of all types, that a few remarks on the methods employed are perhaps advisable. It was found that the discoloration of the cells interfered with the micro-chemical study of the tissues, and it was therefore removed by treatment with eau de javelle. For this purpose the section to be studied was mounted in a drop of that liquid, and heated on the slide over a Bunsen flame. As a rule, from five to ten seconds of this treatment were sufficient to remove all colour. A little fresh eau de javelle was then added to dissolve the crystals formed by the partial evaporation of the first supply, and the whole was then absorbed with filter-paper. The section was next treated with a series of drops of spirit until all traces of the eau de javelle were removed. (About twenty drops successively applied and absorbed were found to work well in practice.) It was then treated with either chlor-zinc-iodine or with phloroglucin followed by either HCl or H_2SO_4 . The walls referred to as delignified invariably gave a positive cellulose reaction with chlor-zinc-iodine, and a negative lignin one with phloroglucin and acid. It appears to be a general impression¹ that treatment with eau de javelle has a delignifying effect on plant tissues, and this is probably the case after more prolonged treatment, but the establishing of efficient controls demonstrated that no error had arisen from this source. In the first place, the whole of the sub-epidermal sclerenchyma remote from the wound and the xylem elements in the stele gave with phloroglucin a positive reaction after treatment, indistinguishable from that given by untreated sections, whilst sections of uninjured *Pteris* petioles, after identical treatment for the short time necessary in these experiments, showed no difference in their reactions. Other control experiments were made with the young stem of *Lycopodium alpinum*, in the thickened cortex of which the lignification is incomplete. In such specimens, treatment with phloroglucin and an appropriate acid produces a pink (ligno-cellulose) rather than a red coloration, and it was found here also that the brief period of treatment with eau de javelle had no delignifying effects. It will thus be seen that the inference that delignification is a traumatic response is amply justified.

¹ Cf. Zimmerman, Botanical Microtechnique : Cavers, Pract. Botany, &c.

The Morphology and Ecology of an Extreme Terrestrial
Form of *Zygnema* (*Zygogonium*) *ericetorum* (Kuetz.),
Hansg.

BY

F. E. FRITSCH, D.Sc.

With three Figures in the Text.

THE Alga *Zygnema ericetorum* (Kuetz.), Hansg. has long been familiar as a form capable of inhabiting both terrestrial substrata and pieces of standing water, but until recently it had received little attention. West and Starkey ('15) have, however, in the present year, added considerably to our knowledge of the Alga in question.

The present communication is mainly concerned with a form of *Zygnema ericetorum* that inhabits certain bare areas on the Hindhead Common (cf. Fritsch and Salisbury, '15, p. 131). The habitat is as inhospitable as could well be imagined and, as a result, the characteristics usually found in the terrestrial form of the Alga are developed to a very pronounced extent. Certain of these characters appear as adaptations to the habitat (see especially the third section of this paper).

Apart from what I will in the following pages refer to as the Hindhead form, I have also examined material of the ordinary terrestrial form from the foot of the scree below the cliffs of Lliwedd on the Snowdon Range, and of the aquatic form from Frensham in Surrey. For the former material I am indebted to Mr. R. J. Tabor, B.Sc., whom I have also to thank for certain details as regards its habitat.

As far as I am aware, only one other member of the Zygnemaceae occurs on terrestrial substrata, and that is *Zygnema javanicum*, v. Martens (De Wildeman, '97, pp. 82, 83), since *Z. pachydermum*, W. and G. S. West, has recently been stated not to be specifically distinct from *Z. ericetorum* (cf. West and Starkey, '15, p. 203). The latter species enjoys a very wide geographical distribution, having been recorded from many different parts of the world, and, in view of its apparent variability, it is not altogether out of the question that *Z. javanicum* may be another form of this ubiquitous Alga.

A. THE CELL-CONTENTS.

The accounts as to the nature of the chloroplasts vary considerably. Schmitz ('88, pp. 18, 44) describes them as closely resembling those of other species of *Zygnema*. Wille ('90, p. 20), in the first edition of Engler and Prantl's 'Natürliche Pflanzenfamilien', speaks of two axile irregular chloroplasts, occasionally coalescing (zusammenfließend) to form an axile strand, whilst in the 'Nachträge' (Wille, '09, p. 11; cf. also Borge, '13, p. 37) he corrects this to read 'one axile chromatophore with one pyrenoid'. Lagerheim ('95, p. 15, foot-note 3) compares the chloroplast with that of *Mougeotia*, and a similar attitude is taken up by Bohlin ('01, p. 51). In their recent paper, West and Starkey ('15) come to the conclusion that in each cell of *Zygnema ericetorum* there is normally only one large axile chloroplast of indefinite outline. 'It is usually constricted in the middle and in some cases twisted. There are two large pyrenoids, one in each half of the chloroplast' (l. c., p. 205). These conclusions are based on a study of carefully fixed and stained material from various localities.

There can be no doubt that West and Starkey's conclusions are true for the ordinary aquatic and terrestrial forms, but they are certainly not applicable to the terrestrial form found at Hindhead. The chloroplasts are, in this case, quite often well defined in the living cell, and can be made to stand out very clearly by treatment with a 8-10 per cent. solution of sodium chloride. In the shorter cells (Fig. 1, G; Fig. 2, D) but a single chloroplast is present, generally with only one pyrenoid. The chloroplasts are, however, quite unlike those figured by West and Starkey, being more or less star-shaped and similar to those of other species of the genus *Zygnema*, although rather more massive, so that the stellate character is less pronounced (Fig. 1). In the longer cells there are often two chloroplasts of this pattern, seemingly quite distinct and, if connected at all, the bridge between them must be exceedingly delicate (Fig. 1, A, B). In other cases these longer cells showed an obvious connexion between the chloroplasts, but it should be pointed out that the individuality of the two portions of the chloroplast was much more sharply marked than is shown in West and Starkey's figures of the ordinary terrestrial form.

Cell-division was only observed in cells containing either two chloroplasts or a chloroplast that was already constricted into two very obvious portions. Each daughter-cell receives one of the two chloroplasts, so that for a time the young cell has but a single chromatophore (Fig. 1, B-D). Sooner or later the chloroplast and pyrenoid gradually broaden out axially (Fig. 3, D) and the pyrenoid divides into two (Fig. 1, E; Fig. 3, E). At this stage, therefore, the cells contain an elongated axial chloroplast with two pyrenoids, more or less widely separated. Finally, the elongated chloroplast becomes gradually constricted between the two pyrenoids (Fig. 1, F)

until in many cells, at the best, but a very narrow connecting bridge remains (cf. above). It would appear that as a general rule the segregation of the two chloroplasts is practically complete before a new septum arises.

A comparison of the above with West and Starkey's account (cf. also Fig. 1 with their Figs. 1 and 2) will show that the Hindhead form differs

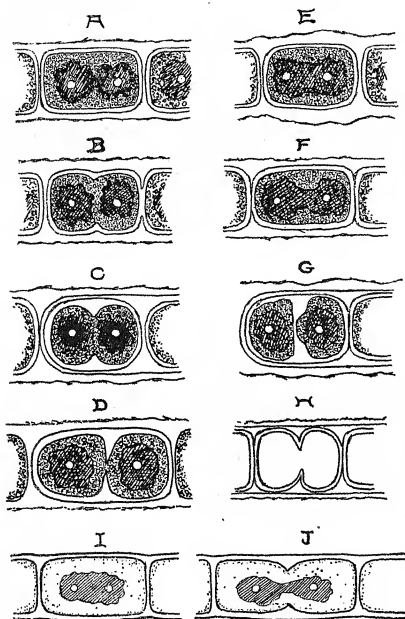


FIG. 1. A-H, cell-structure and cell-division in the Hindhead form of *Zygnema ericetorum*; the drawings made from the living Alga. I-J, cells of the aquatic form, from Frensham; the drawings made from preserved material. For description, see text. (All figures $\times 850$.)

fundamentally from the ordinary type in two respects, viz. in the more definite shape of the chloroplasts, and in the frequent presence of two of them in the mature cell. It may be held by some that the differences are sufficiently pronounced to warrant the establishment of a distinct species, but I am not prepared at present to take up this attitude (see also p. 148). It may well be that observation of forms, like that at Hindhead, is

responsible for the diverse views put forward by different authorities as to the nature of the chlorophyll-apparatus. The chloroplasts of the ordinary species of *Zygnema* are stated to divide at a time when the ingrowth of the septum between the daughter-cells is almost completed (de Bary, '88, p. 11; Wille, '90, p. 17), the young cell thus having two chromatophores except during early stages of division. In this respect even the Hindhead form differs markedly, since doubling of the chloroplasts is delayed until some considerable time after the development of the septum between the daughter-cells or, frequently, until the next division occurs.

The nucleus of the mature cell of the Hindhead form is situated midway between the two chloroplasts and is oval in shape, the long axis being placed parallel to the longitudinal walls of the cells¹ (Fig. 3, C). In the younger cells, with a single chloroplast, it appears to be rounded and apposed to the latter (Fig. 3, D, E) (cf. also West and Starkey, '15, p. 197).

A characteristic feature of the cell-contents of *Zygnema ericetorum* is the presence in the sap of a purple pigment, phycocorphyrin, which has been the subject of a detailed study by Lagerheim ('95). The occurrence of this pigment is, however, somewhat variable. Hassall ('45, p. 175) refers to bright green filaments observed only in aquatic habitats, and purple filaments characteristic of the form spreading over swampy heaths. The aquatic form collected at Frensham, however, had purple sap, whilst part of the material sent to me by Mr. Tabor had little or none, although growing close to, but not intermingled with, other purple-coloured masses, the two apparently being subjected to identical conditions.

Lagerheim ('95, p. 23) has pointed out that it is only the upper portion of a patch, i.e. the part which is strongly illuminated, that develops the purple pigment, and the same observation has been made on the Hindhead form.² This would indicate that the pigment is, at least in part, a protection against strong illumination, and the following appears to lend further support to this view. At one point the Hindhead form is becoming overgrown by bright green filaments of a Ulotrichaceous Alga, that seems to correspond to *Hormidium flaccidum*, A. Br. (Fritsch and Salisbury, '15, p. 131). An examination of the underlying filaments of the *Zygnema* shows that the chloroplasts have shifted from the middle to the upper sides of the cells, so that these appear green, in contrast to the purple-coloured under sides. It seems that, as a result of the screen formed by the *Hormidium*, the chloroplasts of the underlying Alga do not receive sufficient light, a disadvantage remedied by their moving to the upper sides of the cells. A patch of the typical purple-coloured Hindhead form was

¹ I am much indebted to my colleague, Dr. Salisbury, for kindly microtoming material of the Alga.

² The green filaments from the under side of the patch are excellent material for demonstrating the two massive chloroplasts of the mature cell.

placed in the laboratory under a screen formed by a thick pad of cotton-wool; after three days a considerable number of the threads showed a similar shifting of the chloroplasts to the upper sides of the cells, so that this movement is evidently readily accomplished.

The possibility of the pigment being related to the low temperature of the habitat was also considered by Lagerheim, but, in view of the abundant occurrence of *Zygnema ericetorum* in the Tropics, this explanation does not seem very plausible. On the other hand, as Bohlin ('01, p. 51) suggests, its appearance may be connected with the extremes of temperature to which such a terrestrial Alga would be subjected. The phycoporphyrin may also be related in some way to the remarkable power of resistance to desiccation possessed by this Alga (cf. p. 145).

B. THE CELL-WALL.

The cell-wall of the Hindhead form is thick and stratified, these features being more pronounced than is usual in the terrestrial form. Two or three regions are generally distinguishable in the longitudinal walls. The innermost layer is well defined, appearing in optical section as a bright line of varying thickness. Beyond this come successive strata, making up the greater part of the wall and forming what I will call the intermediate layer. These strata, which are not always easily recognized individually (although plainly seen in filaments placed in strong potash), appear progressively duller and duller the further out they lie. The manner of refraction of the light shows that the innermost layer is the densest, and that from there outwards the layers decrease in density, probably owing to gradual gelatinization (see below). In some cases the surface of the filament is quite smooth, but as a general rule the latter is bounded by a much interrupted dark line, composed of numerous adhering foreign particles (Fig. 1). There is often more or less obvious constriction between the cells. On treatment with dilute sulphuric acid, the surface and intermediate layers swell and become lost to view, whilst the inner layer remains distinct for some little time longer. Addition of iodine, subsequent to the acid, gives the blue coloration of cellulose (cf. West and Starkey, '15, p. 194).

The same three layers are recognizable in the aquatic and ordinary terrestrial forms, but in both the intermediate layer is much thinner and, even in the latter, stratification is often unrecognizable without the application of special means. The surface is quite smooth.

The Hindhead form, if collected during a period of drought, consists of a hard brittle mass having an almost horny consistency. If a small portion be placed in a drop of water, it *instantaneously* softens and becomes exceedingly pliable. When a larger piece is brought into contact with water, swelling of the Alga is macroscopically visible, and if the quantity of water be small, the bulk of it is *immediately* absorbed, far more rapidly

than by a piece of filter-paper of equivalent size. These phenomena are due to the fact that the greater part of the thick wall is mucilaginous, so that the dry filament swells up very considerably on wetting. Thus, the average width of the dry threads is 16μ , of the wet threads 24μ ; the average thickness of the longitudinal walls of dry filaments (examined in spirit) is 2.3μ , of wet filaments 5μ .

It does not appear that the inner layer of the wall is at all appreciably mucilaginous, since it retains its outline and normal appearance, even in dry threads. On the other hand, the intermediate layer must be very largely gelatinous in character in the Hindhead form, as it is very much shrunken and often quite thin in the dry filament. In all probability the mucilaginous character increases from the interior towards the outside, the surface layer being very strongly mucilaginous (cf. above).

The advantage of this mucilaginous wall to the Hindhead Alga is obvious. It not only involves a very rapid recovery of the cells, when supplies of moisture become available, but, since the gelatinous walls will only part with their water by slow degrees, the full effects of a drought will only be felt by the Alga some time after dry weather sets in. It is indeed astonishing how long the desiccated filaments still retain their soft flexible character. Moreover, at certain periods of the year, although exposed to intense insolation in the daytime, such a form will be able to make use to the full of the dew deposited at night, so that even during periods of drought it may be able to hold its own to some extent. In short, we see in this character a pronounced adaptation to the conditions of the habitat. In the material of the ordinary terrestrial form at my disposal, the intermediate layer was generally much thinner and did not appear to be appreciably mucilaginous.

A considerable number of aquatic Zygnemaceae form gelatinous sheaths about their filaments, but these are sharply marked off from the cell-wall, and there is reason to believe that they are composed of mucilage excreted from the cell-contents (Klebs, '86, p. 368) and not, as in the Hindhead form, a product of the membrane. West and Starkey ('15, p. 194) record the presence of an occasional more or less distinct gelatinous sheath in *Z. ericetorum*, but I have never met with anything of the kind in the Hindhead form. Thick and stratified mucilaginous membranes are, on the other hand, frequently found in the resting-cells (akinetes) formed, as a result of desiccation, by many species of *Zygnema* (*Z. leiostermum*, *Z. pectinatum*) (de Bary, '58, pp. 9, 10), so that it might be said of the Hindhead Alga that it existed permanently in the akinete condition.

The development of the septum between two daughter-cells is initiated by an annular invagination of the innermost layer of the wall, about midway between the two chloroplasts or the two halves of the chloroplast (Fig. 1, B). It often has the appearance as though this were due to

a gradual thickening of the intermediate layer pushing in the inner layer at this point. The invagination slowly progresses towards the centre (Fig. 1, C, D), thus gradually constricting the protoplast in the manner customary among the simpler Algae. It does not seem, however, that a complete septum is at once formed, the two daughter-cells remaining for some time connected by a more or less narrow pore (Fig. 1, D, H), which probably ultimately closes. In optical section the continuity of the two protoplasts is often readily recognized. The septum at its first initiation is not a uniform structure, but exhibits three layers meeting in a point at the inner edge of the annular invagination. Two of these layers, forming the limbs of a V, are continuous with the innermost layer of the longitudinal wall, whilst the third lies between them and appears to be similar in nature to the intermediate layer (cf. especially Fig. 1, H).

In many of the filaments of the Hindhead form one observes pairs of daughter-cells in which the one protoplast is prominently beaked towards that of the other cell, even when seen in surface view (Fig. 1, G; Fig. 2, C). This phenomenon is a result of the frequent asymmetry of the ingrowing septum. The latter, in optical section, either appears symmetrical and V-shaped (Fig. 1, B, C) or its two edges are asymmetrical (as in Fig. 1, D, G), the one being generally placed at right angles to, the other at an obtuse angle to, the longitudinal wall. It has not been possible to find any underlying reason for the development of these asymmetrical septa, which are very commonly observed.

C. THE REACTION TO DROUGHT ON THE PART OF THE HINDHEAD FORM (FIG. 2).

The appearance of a mature filament of the Hindhead form is a definite expression of the alternating periods of drought and rainfall which follow one another in the habitat. On the arrival of a dry period the protoplast, probably shrinking slightly and mostly rounding off to a more or less oval or spherical shape, according to the previous form of the cell, generally excretes a new layer of membrane on its outer surface and contiguous to the inner layer above described (p. 139). The latter often seems at the same time to lose in definition and gradually to become merged in the intermediate layer, but this does not necessarily occur; particularly in thick-walled filaments the former inner layer may persist for some time as a definite stratum in the intermediate layer.

The septa also generally lose their sharp definition and, as the new membrane develops on either side, the substance composing them becomes gradually indistinguishable from the intermediate layer of the longitudinal walls, which now therefore extends uniformly all round the cell (Fig. 1, A; Fig. 2, F). Except for the development of the new layer of membrane and

the moderate rounding off of the protoplast, no changes seem to be connected with the production of these 'akinetes' (cf., however, below); the chloroplast or chloroplasts, as the case may be, are still recognizable and

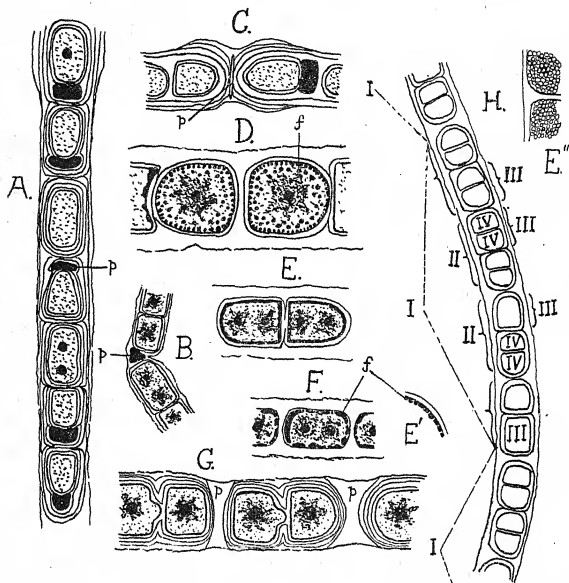


FIG. 2. Terrestrial form of *Zygnema ericetorum* from Hindhead. A, formation of akinetes and pigment-cells (the latter in black). B, impending rupture of a filament at a point where a pigment-cell is situated. C, obliteration of an empty pigment-cell by growth of adjacent akinetes. D, ordinary cells of the Hindhead form, showing the disposition of the fat-globules. E, cells of a filament that has been subjected to desiccation, showing the dense peripheral layer of fat-globules. E', small part of one of these cells, in optical section. E'', ditto, in surface view. F, cells of a dry filament, showing the large peripheral fat-masses that are sometimes found. G, filament, showing divided akinetes and empty pigment-cells. H, diagram of a filament showing the products of successive akinetes, I, II, III, IV. p, pigment-cell; f, fat. (B and H, $\times 400$; A, C, E, E', F, $\times 650$; D and G, $\times 850$; H $\times 1200$.)

the purple-coloured sap appears unaltered. The response to drought is thus of a very simple kind.

With a return of more favourable conditions, moisture is rapidly absorbed, as described above. The filament immediately resumes its normal appearance, and cell-division sets in sooner or later. If the akinete

was produced from a mature cell with two chloroplasts, a formation of daughter-cells may take place almost immediately. If, on the other hand, the akinete was formed soon after a cell had divided, so that it contained but one chloroplast, division may be considerably delayed. The septa cut the akinetes into two approximately equal halves and, since the adjacent faces of the daughter-protoplasts are more or less flat, whilst their distal faces are often rounded off, the pairs of protoplasts produced from a single akinete, as the result of division, frequently recall the two halves of a *Cosmarium*-cell (Fig. 2, H). In this way the products of division of the individual akinetes of each filament are quite plainly distinguishable.

The two daughter-cells may divide once more, this depending on how soon the first division sets in after the arrival of favourable conditions. Thus we may get groups of four cells (Fig. 2, H, II) developed, before a fresh drought causes renewed rounding off and fresh akinete formation. I have never observed more than four cells thus produced from a single akinete, during the interval between one drought and another.

The repeated formation of akinetes, involving each time the apposition of a new layer of membrane on the inner side of the cell-wall, leads to a gradual thickening of the latter, mainly noticeable in the transverse septa. In examining a given length of filament, strongly thickened septa are found at more or less remote intervals (Fig. 2, H, I). Between these one meets with septa of varying thickness, some quite thin and recently formed, others already more or less thickened and of older date (Fig. 2, H, II, III, IV). The region lying between two strongly thickened septa is the product of a single akinete of a remote generation; the intervening cells can be grouped, on the same principle, into smaller and smaller sets formed from more and more recently produced akinetes. In this way, by a careful scrutiny, it is often possible to estimate roughly the number of periods of drought to which a given filament has been subjected (Fig. 2, H). The longitudinal walls do not appear to increase much in thickness beyond a certain stage, since the outermost layers gradually merge into mucilage (cf. above).

In the cells of the filaments of the Hindhead form, numerous small globules, of irregular shape and varying size, are found distributed, more particularly in the peripheral part of the protoplast (Fig. 2, D). I have not observed such globules in the aquatic form, and in the terrestrial form from Wales they occur in much smaller numbers and are, on the average, much coarser (cf. also West and Starkey, '15, p. 197). When threads of the Hindhead form are subjected to gradual desiccation, an exceedingly dense layer of small and rather uniform globules appears in immediate contact with the inner layer of the cell-wall, whilst relatively few are found in the rest of the protoplast (Fig. 2, E, E'). The peripheral layer of globules is so dense and so closely apposed to the cell-wall that it looks like a second

pitted membrane in optical section (Fig. 2, E') and gives a characteristic mottled appearance to the protoplast in surface view (Fig. 2, E''). If such dried filaments are kept in water for a few hours, this distinctive arrangement of the globules becomes less pronounced. They no longer form such a dense continuous layer in all the cells, nor are they quite so closely adpressed to the cell-wall, although they still occupy in the main the peripheral protoplasm (Fig. 2, D). The filaments, subjected to dryness, when first placed in water, appear more transparent than after being immersed for some time, and this loss in transparency is probably due to the much more irregular distribution of the globules in the second case. Not only does the arrangement of the globules become less regular in filaments immersed in water, but it appears that they also undergo increase in size, perhaps accomplished by coalescence of the smaller ones. Lastly, it was found that in threads which had been subjected to prolonged drought, the globules in many of the cells were partly replaced by one or more highly refractive masses of considerable size, again located in the periphery of the protoplast (Fig. 2, F).

De Bary ('58, p. 10; Pl. I, Figs. 16, 20) has already described such globules in the resting-cells of other species of *Zygnema*, and refers to them as fat-bodies. Their reactions with osmic acid and tincture of alkanna, as well as their solubility in chloroform, are quite in accordance with this view (cf. West and Starkey, '15, p. 198). De Bary does not deal with the varying disposition of the fat-globules in dry and moistened filaments, but, seeing that these resting-cells are very similar to those of the Hindhead form, it is probable that they might show the same phenomena.

When cells, whether of the dry or wet filaments, are plasmolysed by immersion in a strong solution of sodium chloride, the globules in question become altogether lost to view. When the plasmolysed cells are allowed to recover by placing the threads in water, the globules again become visible; in many of the cells they do not appear to have undergone any change, but in some they are not quite as densely arranged as before. Plasmolysis does not appear to be effected by anything less than an 8 per cent. solution of salt, and, with this strength, but a very slight separation of the protoplast from the cell-membrane takes place. In stronger solutions (e. g. 10–12 per cent.) some of the cells become more markedly plasmolysed, a very obvious contraction of the protoplast taking place, but it requires a 15 per cent. solution to produce marked plasmolysis in *all* the cells. As far as I have been able to determine, there is no difference between the dry and wet filaments in these respects. Attention may be called to the very high osmotic pressures manifested by the cell-sap in this *Alga*.

The fact that the peripheral layer of fat-globules remains more or less intact, after recovery from plasmolysis, would indicate a fair degree of stability. Whenever there is a shrinkage of the protoplast, such as probably

occurs to a slight extent when the threads are exposed to drought, the smaller surface area of the-protoplasm must lead to a still closer crowding of the peripheral layer of fat-globules. If we could imagine that this contraction brought about a coalescence of the individual fat-bodies, a continuous layer of fat would be formed on the surface of the protoplast and this would act as a kind of macintosh to the latter, tending to prevent evaporation from the cell. But such a coalescence is unlikely on physical grounds, and moreover, if it occurred, it would be difficult to understand how the peripheral layer came to consist of numerous minute separate globules the moment the dry filament was placed in water. For the present, therefore, the function of this peripheral fat-layer must remain unsolved, although its extreme development, especially in the cells of the dry threads, will dispose one to associate it in one way or another with the great power of resistance to drought possessed by the form under discussion.

This power is displayed not only by the instantaneous recovery of the filaments, when placed in water, even after many months of desiccation, but also by the fact that there does not appear to be any appreciable difference in osmotic pressure between the cells of dry filaments and of those growing under moist conditions. Material collected at Hindhead was kept for five months in part dry, in part moist. At the end of this time it was found that there was no difference between the two sets as regards the number of living cells in the threads.

According to Lagerheim ('95, p. 24) the cells of the filaments of *Z. ericetorum*, on being subjected to desiccation, become filled with reserve substances and thicken their walls, whilst the phycoporphyrin disappears; as a consequence the resting-cells are almost colourless. In the case of the Hindhead form, although it has been collected at many different times of the year, such akinetes have never been observed; in fact, the cells of the resting threads are coloured just as deep a purple as those of the active filaments. It is plain therefore that the state of affairs described by Lagerheim is not an essential consequence of drying up.

On the other hand, a modification of the ordinary response to desiccation has been observed in material of the Hindhead form collected in the early part of the year, and here a certain reduction in the amount of pigment may possibly take place. In this case, the contents of most of the cells had divided unequally into a large akinete and a small cell, apparently provided with relatively little protoplasm, but filled with deep purple sap; the pigment-cell formed a kind of cap over the end of the adjacent akinete and was cut off from it by a delicate wall (Fig. 2, A). The pigment-cells were either produced on the same side over a considerable length of filament or no such regularity was apparent (Fig. 2, A) (cf. West and Starkey, '15, p. 199, Fig. 2, c). As a general rule the akinetes were more or less pointed

towards the pigment-cells, whilst their distal ends were rounded or almost truncate (Fig. 2, A), and it will be noticed that these akinetes were provided with an especially thick and stratified membrane.

Since this mode of akinete formation was observed mainly in winter, it is possible that the extrusion of purple sap into the pigment-cell, by reducing the water-content of the protoplast of the akinete, renders it more resistant to frosts, to which in the exposed habitat it must be very liable. This explanation is, however, only advanced tentatively, since the phenomenon may be due to other causes, but attention should be drawn to the

fact that it was observed in the bulk of the filaments over a considerable area. On a much smaller scale it has also been encountered at other times of the year, but only with reference to occasional akinetes.

As above indicated, the division leading to the production of akinete and pigment-cell is an unequal one and due to the development of a septum, generally of the asymmetrical type (p. 141), towards one end of the cell. In fact, the asymmetry of the septum is responsible for the usual pointed shape of the akinete at the end adjacent to the pigment-cell. When renewed growth takes place, the contents of the latter gradually disintegrate and disappear, so that sooner or later the pigment-cell is quite empty (Fig. 2, G).

These empty cells constitute so many weak points in the filaments, at which rupture readily takes place, and therefore this mode of akinete

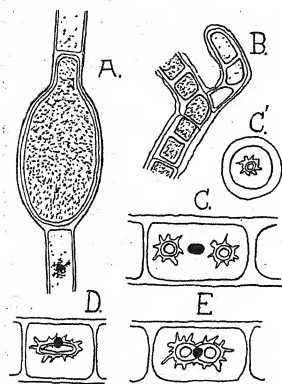


FIG. 3. A, swollen akinete of terrestrial form from Wales. B-E, terrestrial form from Hindhead. B, rhizoid-formation. C-E, cells from microtomed material of the Hindhead form; C-E in longitudinal section; C' in transverse section. C, a mature cell. D and E, stages in division of the chloroplast. The nucleus is shown black. (A and B, $\times 400$; C-E, $\times 650$.)

formation probably brings with it the advantages of extensive vegetative propagation (cf. Fig. 2, G). As a matter of fact, many of the filaments of the Hindhead form terminate in short empty fragments of cell-membrane appearing just like the ruptured remnants of pigment-cells. In some cases, however, the adjacent akinetes, on resuming growth, protrude from either side into the empty pigment-cell, so that the cavity of the latter becomes more or less obliterated, and ultimately appears only as a narrow slit in the thick membrane between the two (Fig. 2, C).

The dying away of occasional cells of a filament is not confined to

these special pigment-cells, being not uncommon at all times of the year (cf. West and Starkey, '15, p. 198). The cells in question generally appear deeply pigmented at first and, as indicated in Fig. 2, B, constitute points at which the filaments readily give way. In many cases rupture is delayed until the contents have completely disappeared. Apart from such isolated cells, it occasionally happens that large numbers of the cells of a thread die away, leaving healthy akinetes only here and there, separated by more or less extensive stretches of dead filament. The akinetes in such threads are generally well rounded and provided with a very defined layer of the peripheral fat-globules. No doubt they ultimately become free and give rise to new threads.

Mention may be made at this point of a peculiar form of akinete, rarely observed in the material from Wales (Fig. 3, A). In this case the greater part of the cell had swollen up very considerably, this distension however not affecting the last quarter of the cell, so that the resulting shape rather recalled the oogonia of some *Oedogoniums*. The wall was rather strongly thickened and stratified, and the cell-contents, though appearing perfectly healthy, were so dense as to make it impossible to decipher any details of structure. Nothing corresponding to these swollen cells has been observed in the remaining material. Apart from such elements, no akinete formation was observed in the material from Wales.

D. COMPARISON OF THE HINDHEAD FORM WITH THE AQUATIC AND ORDINARY TERRESTRIAL TYPES.

Apart from the chloroplasts which have already been fully considered (p. 136), the Hindhead Alga differs from the ordinary terrestrial form of *Zygnema ericetorum* in the length of the cells, the degree of thickening of the walls, and the abundance of the fat-globules. The following table shows the relative dimensions of the Hindhead form, the form from Wales, and the aquatic one:

	Terrestrial form, Hindhead.	Aquatic form, Frensham.	Terrestrial form, Wales.
Length of cell	26.5 μ (24-30) ¹	40 μ (34-48)	31.1 μ (21-48)
Width of cell	25.5 μ (24-27)	19 μ (16-21)	24.2 μ (23-26)
Thickness of longitudinal walls	5 μ (4-6)	1.8 μ (1.5-2)	3 μ (2-3.5)

It should be mentioned here that the form from Wales is perhaps not quite typical of the true terrestrial Alga, since it occurred in the immediate neighbourhood of numerous small streams which are practically never dry. As contrasted with the conditions obtaining at Hindhead, such a form would be very advantageously situated. It can scarcely be doubted that the extreme shortness of the cells of the Hindhead form is a result of the brief periods during which growth is alone possible.

¹ The figures in round brackets give the extreme dimensions.

Two other features are generally cited as characteristic of the terrestrial form, viz. the frequent swollen shape of the cells and the formation of short rhizoids (Borge, '18, p. 37; Collins, '09, p. 120). As regards the first point, I have been unable to find any marked difference between the three forms in the shape of the cell, which varies between cylindrical and somewhat barrel-shaped. The rhizoids, which may be uni- or multicellular (Fig. 3, B), have been encountered only in the two terrestrial forms.

To raise once again the question of the possibility of the Hindhead Alga being a separate species, it may be pointed out that, except for the chloroplasts, all the distinctive features appear merely as intensifications of the characters of the ordinary terrestrial form. As for the chloroplasts, the greater degree of independence of the two halves may be a result of the very slow growth of the Hindhead form and the consequent long intervals between successive cell-divisions. In short, I am inclined to regard the Hindhead Alga merely as a very extreme xerophytic form of *Zygnema ericetorum*.

E. SUMMARY.

An extreme terrestrial Alga is described, probably belonging to *Zygnema ericetorum* and owing its peculiarities to the extremely inhospitable habitat on the Hindhead Common. The mature cell of this form contains two chloroplasts, much like those of other species of *Zygnema*, although they may hang together by an exceedingly narrow connecting bridge. For some time after division but a single chloroplast is found in each daughter-cell. Division is accomplished by the gradual invagination of the innermost layer of the cell-wall, but it appears that the septum thus formed is not completed for some time, a central pore remaining through which the daughter-protoplasts stand in connexion with one another. Two or three layers are distinguishable in the longitudinal walls.

The Hindhead Alga may be said to be permanently in the akinete condition, its cells agreeing in many respects with the akinetes of such forms as *Z. leiosperrum*, *Z. pectinatum*, &c. The outer portion of the wall is strongly thickened and mucilaginous, and is shown to play a great part in protecting the cells during periods of drought and in bringing about a rapid recovery on the reappearance of favourable conditions. The cells contain numerous fat-globules (Fig. 2, D), which, on the commencement of desiccation, form an exceedingly dense layer closely apposed to the inner surface of the membrane (Fig. 2, E, E'); the function of these globules is not exactly clear. Some hours after moistening the dry filaments, the regular peripheral disposition of the fat-globules more or less disappears.

With the advent of a dry period the protoplasts round off slightly and develop a new layer of membrane. The products of division of successive akinetes (i.e. the growth during the intervals between two periods of

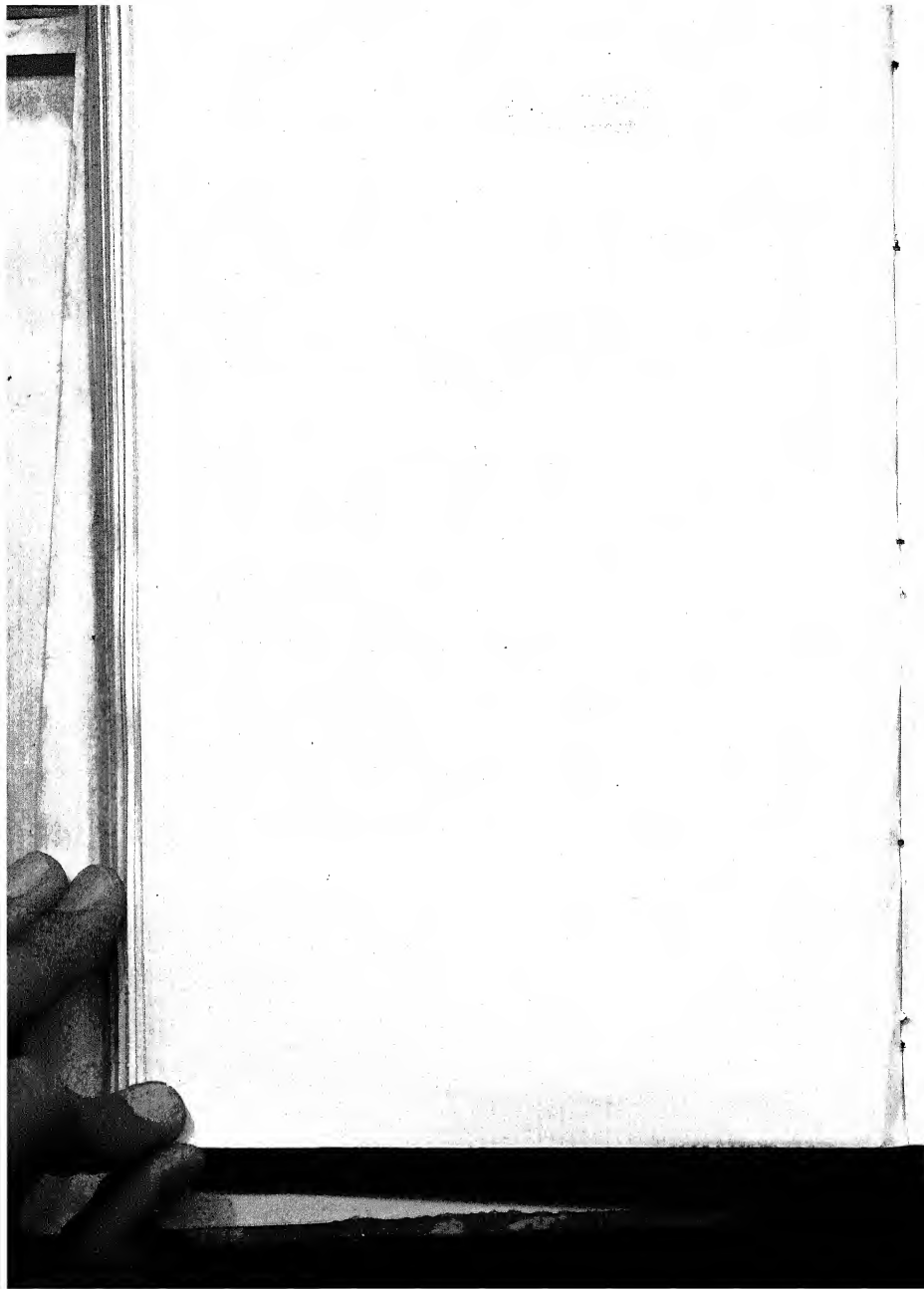
drought) are plainly distinguishable and show that, as a general rule, each cell divides at the most but twice between two successive dry periods (Fig. 2, H). Apart from the ordinary mode of response to drought, a second method of akinete formation has been observed in the early part of the year; in this the cells undergo unequal division, resulting in the formation of an akinete and of a much smaller pigment-cell (Fig. 2, A). The contents of the pigment-cells subsequently disappear, and the empty cells form weak points at which rupture of the threads readily occurs.

Attention is drawn to the extreme adaptation of this form to drought. The recovery of the dry filament is practically instantaneous when placed in water, there is no marked difference between the osmotic pressure of moist threads and those subjected to dryness, and there is no greater percentage of dead cells in filaments that have been kept dry for months than in those that have been in water for the same length of time.

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EAST LONDON COLLEGE,
November 1, 1915.

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Dysmorphococcus variabilis, gen. et sp. nov.

BY

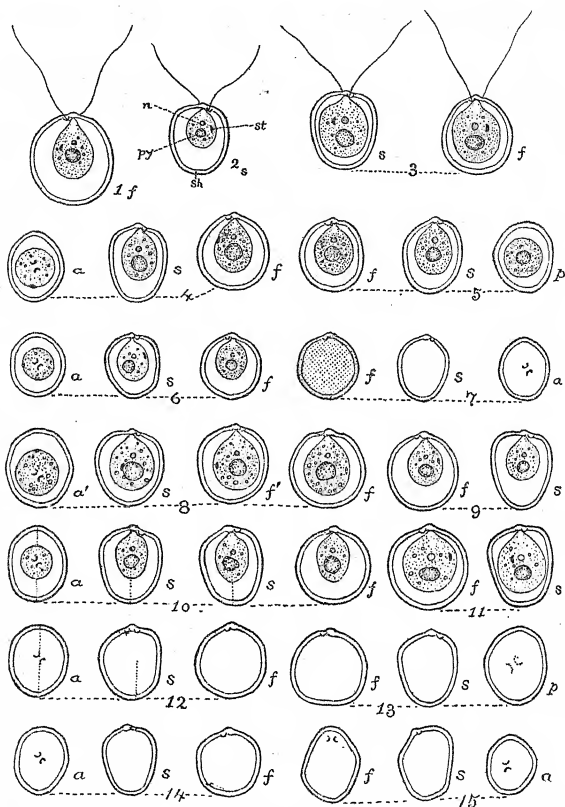
H. TAKEDA, D.I.C.

With fifteen Figures in the Text.

THE Alga which is here described under the name of *Dysmorphococcus variabilis* was found in gatherings made during October, 1915, in a small stagnant pond in Richmond Park, Surrey. It occurred sparingly, associated with some species of *Closterium*, *Cosmarium*, *Chlamydomonas*, *Carteria*, several species of *Euglena* (e.g. *E. oxyuris*, Schmarda, *E. spiroyra*, Ehrb., &c.), *Lepocinclis* (e.g. forms of *L. ovum* (Stein), Lemm., *L. Steinitz*, Lemm., &c.), *Phacus* (e.g. *Ph. caudata*, Hübn., *Ph. longicauda* (Ehrb.), Duj., and its var. *torta*, Lemm., *Ph. parvula*, Klebs, *Ph. pusilla*, Lemm., forms of *Ph. pyrum* (Ehrb.), Stein, *Ph. tenuis*, Swirenko, *Ph. triquetra* (Ehrb.), Duj., &c.), *Trachelomonas* (e.g. forms of *Tr. hispida* (Perty), Stein, *Tr. rugulosa*, Stein, *Tr. Stokesiana*, Palmer, forms of *Tr. volvocina*, Ehrb., &c.), and *Vacuolaria virescens*, Cienkowski.

Externally the organism under consideration very much resembles the small subglobose form of *Trachelomonas volvocina*, Ehrb. It can, however, at once be distinguished from the latter, even under a low power of the microscope, by the fact that while it swims about, rotating on its own axis, and, so far as ascertained, always forwards, it presents a more or less angular appearance, due to its irregular shape. An examination under a high power reveals the fact that the organism does not belong to this genus, although there is at least one species (probably new) of *Trachelomonas* having a shell compressed in a somewhat similar way.

The organism in question possesses a hard, brittle shell, similar in texture to that in the majority of species of *Trachelomonas*, brown to dark brown in colour, and extraordinarily irregular and variable in shape. The shell easily cracks and breaks up into irregular pieces when subjected to slight pressure, or when fairly strong glycerine is added to the water under the cover-slip. The shell is about 1μ in thickness, and is ornamented with very minute and regularly arranged granules which resemble the dots in a half-tone print (Fig. 7, f). These granules are in some cases very faint, yet can always be detected, at any rate in all the specimens examined. It



Figs. 1-15. *Dysmorphococcus variabilis*, Tak., gen. et sp. nov. All $\times 1,000$. a = anterior view; a' = oblique anterior view; f = front view; f' = oblique front view; p = posterior view; s = side view; n = nucleus; py = pyrenoid; sh = shell; st = stigma (= eye-spot). Figs. 7, 12-15 represent empty shells. Stigma is not represented in Fig. 10, which was drawn from a specimen the colour of which had faded after fixation and mounting.

can be positively stated that they are granules, and not scrobiculations. It may also be mentioned here that the shell usually causes the interference of light on its margin, in the same way as in many smooth species of *Trachelomonas*.

In the front view the shell appears as a rule more or less round or broadly ovate (*f* in Figs. 1, 3-12). In rarer cases it is more or less quadrangular (*f* in Figs. 13, 14), and very seldom it is irregularly rhomboidal (Fig. 15, *f*). In the side view it is more or less pentagonal in form, narrowing towards the base. The end views (*a* and *p* in Figs. 4-8, 10, 12-15) are irregularly oval, and show two ciliary apertures. The part of the shell between the two apertures projects very slightly, forming a very small beak-like protuberance.

In many cases, especially in the specimens with distinct granules, a faint yet conspicuous line (dotted) on the long axis of the shell in both side and end views is noticeable (cf. *a* and *s* in Figs. 10, 12). This dotted line extends round the base of the shell, but gradually disappears when the upper region of the shell is reached. The only explanation I can offer at present is that this line is due to a coalescence of some of the granules on the surface of the shell. It is probably brought about by the fact that as the shell gradually gets narrow towards its base, the granules, which are, as already described, very regularly distributed, find in the lower region of the shell less room for their arrangement, consequently some of them may be expected to become confluent. This is assumed to have occurred along the median line, resulting in the production of the dotted line above referred to.

This probably accounts for the fact that the dotted line is always circumbasal, but never encircles the whole shell.

As to the question whether this dotted line indicates that the shell consists of two more or less equal halves and that the line may be regarded as a suture, I am not prepared to give an answer. Only once a piece of shell was found, which had been apparently broken along this line, but this isolated occurrence may perhaps have been accidental.

The ciliary apertures above referred to are very minute, being just large enough for the necessary play of the flagella, and have no thickening on the margin. It seems that they are more or less funnel-shaped, with the narrow end directed towards the apical beak of the protoplast, to which the flagella are attached. On this account, it usually happens that the apertures are viewed more or less obliquely, and they then appear as semicircles.

The apertures lie in a plane which is not parallel to the long axis of the anterior end view of the shell, but cuts the median line at an angle of about 45° (cf. *a* in Figs. 7, 8, 10, 12, 14, and *p* in Fig. 13), so that each half of the shell appears to have one of the apertures belonging to it. For this reason, only one of the apertures is distinctly visible in either the front

view or the side view, except in the case of an extremely irregular specimen, such as that delineated in Fig. 15. The direction of the plane, including the apertures, is practically constant, hence in the front view the aperture is seen on the left-hand side of the beak, and in the side view on the right (compare the front and side views with the end views).

The size of the shell varies as much as the shape does. Fig. 1 represents the largest specimen I have examined, and Fig. 7 the smallest. The range of size is therefore 14–19 μ high, 13–17 μ broad (i. e. in the front view), and 10–14 μ thick (as seen in the side view).

The actual body of the Alga inside the shell is often considerably smaller than the shell, a clear space being present between them. In all the specimens examined the body never completely fills up the shell. The space is an actual one, and is not homologous with the so-called 'space' found in *Sphaerella*, *Chlamydomonas*, *Carteria*, and some other allied genera, in which the 'space' is really the gelatinous part of the cell-wall. It is probable that the space in *Dysmorphococcus* contains water in which the organism lives. In one case I observed a living specimen, the shell of which became accidentally cracked and broken, presumably by pressure of the cover-slip. The organism, which had been vigorously swimming, did not seem to suffer at all, and as soon as a little water was added, which enabled the monad to move more freely, it resumed its propulsion and actively swam with the broken shell hanging on to the body.

The protoplast is roughly pear-shaped, tapering towards the apex into a short colourless beak (Figs. 1–6, 8–11). At this end of the protoplast the algal body is apparently in connexion with the apex of the shell. Two flagella of equal length are attached to the beak, and emerge through the apertures above described. So far as could be ascertained, the protoplast has no definite cell-wall,¹ but, unlike that of Polyblepharidae, it does not show amoeboid movements. There is a single chloroplast which is urceolate and occupies practically the whole of the body. In most cases the chloroplast is pale green and contains a few minute granules, the nature of which has not been determined. In one case (Fig. 8) a larger number of granules was observed, and this character, like the occurrence of an exceptional colour in the chloroplast of many *Chlamydomonads*, is probably due to special physiological conditions. A small discoidal stigma (eye-spot) is present, and has a peripheral position, being external to the chloroplast and forming a slight prominence on the surface of the protoplast (α in Figs. 4, 6, 8). Its position varies to some extent; in some specimens it occurs in the middle portion of the body, while in others it is nearer the apical beak. A pyrenoid of fairly large size is always present near the base of the chloroplast, or in some cases at a slightly higher level. The pyreno-crystal and amylaceous envelope can easily be differentiated by means of the usual stains, including

¹ The shell is not regarded as a cell-wall.

iodine. The nucleus, which is very small, is situated at the bottom of the central protoplasmic mass within the hollow of the chloroplast. So far no contractile vacuoles have been observed.

As already mentioned, the protoplast varies in size, being as a rule much smaller than the shell, but sometimes almost filling up the lumen of the latter. The smallest protoplast observed measures 8μ in length and 6μ in transverse diameter, while the largest one reaches 12μ in length and 10μ in transverse diameter. The variation in size of the protoplast does not run parallel with the size of the shell; thus a small shell may be found to possess a protoplast of relatively large size, while a large shell may contain a small protoplast. The variations in the size of the protoplast are probably connected with its growth, the proportional size of the protoplast being presumably an index of the age of the specimen.

It is much to be regretted that we have no knowledge regarding the reproduction of this Alga.¹ It may, however, be presumed that the protoplast, when it has reached a certain size, divides into possibly two or four, and gives rise to daughter-cells within the original shell. Hence the examples with a relatively large protoplast may perhaps be regarded as a stage preceding asexual reproduction. At a certain stage after division of the protoplast into daughter-cells, the shell no doubt breaks and liberates the young naked individuals, each of which sooner or later must secrete a shell for itself. It seems improbable that the daughter individuals become provided with shells within the mother-shell.

Since the life-history of this Alga is not known, nothing precise can be stated as to the affinity of this peculiar organism. However, so far as the vegetative characters are concerned, *Dysmorphococcus* undoubtedly possesses all the essential features of the Volvocaceae. Amongst the described members of this family, the organism under consideration appears to be closely related to *Coccomonas*,² which, however, differs in having a definite cell-wall and a large single aperture in the shell for the emergence of both of the flagella. For the present it may be convenient to place our new genus in the Phacoteae, a sub-family of the Volvocaceae. Since this new alga possesses two ciliary apertures in the shell, it may be suggested that the organism should be compared with *Isococcus*,³ which has been described as having an envelope of a similar nature. An examination of the original preparations of *Isococcus* has convinced me that there is no relationship

¹ No stage of division has been found either in nature or in the laboratory cultures, the latter having been not very successful. As the author is returning to Japan shortly, it is impossible for him to continue his investigations. Further study of this singular organism, in particular with regard to reproduction, is therefore left to the hands of those botanists who are in a position to obtain fresh material. The pond in which the organism was found lies near the south-eastern corner of Conduit Wood and can easily be reached by entering the Park through Richmond Gate.

² Cf. Wille, in Engler and Prantl, *Pflanzenfam.*, I. 2, p. 40, Fig. 22, M, N (1897), and also his *Nachträge* to Chlorophyceae (1909).

³ Fritsch, in *New Phytologist*, vol. xiii, p. 341 (1914).

whatsoever between these two organisms, except in so far as they both belong to the Volvocaceae. As a reinvestigation of *Isococcus*, in conjunction with Professor F. E. Fritsch, is in progress, further remarks upon this organism will be dealt with later in a separate paper.

DIAGNOSIS.

Dysmorphococcus,¹ Tak., gen. nov. Cellulae vegetativae nudaе, libere natantes, polo apicale in rostrum brevissimum decoloratum producto et flagellis binis aequilongis quam corpore cellulae sesqui- vel subduplo longioribus praedito, in tegumento rigido fragilique, brunneo, plerumque valde distento, aperturis binis instructo, nascentes. Chromatophora singula, viridis, urceolata; stigma parvum, parietale; nucleus fere centralis vel paulo anterior; vacuolae contractiles carentes(?). Propagatio ignota. Grege *Coccomnadis* collocandum esse videtur, sed a qua tamen membrana cellulae destituta aperturis flagellorum binis differt. Species unica.

Dysmorphococcus variabilis, Tak., sp. nov. (Figs. 1-15). Cellulae pyriformes, stigmate in parte media vel subantere, pyrenoide singulo basali vel submedio, subconspicuo; tegumentum, brunneum vel atrobrunneum, granulis minutissimis regulariter distributis ornatum, valde polymorphum, a fronte visum rotundato-ovatum, raro subquadrangulare, rarissime irregulariter rhomboidale, a latere visum pentagonum, basin versus subcuneatum plus minus attenuatum, a vertice visum subovale, utraque aperturas flagellorum minutas, margine non incrassatas manifestans. Tegument. long. 14-19 μ , lat. 13-17 μ , crass. 10-14 μ .

Hab. In stagno prope silvam Conduit Wood, in Richmond Park, Surrey (Oct., 1915)

I take this opportunity of expressing my sincere thanks to Professor G. S. West, of Birmingham, for his helpful suggestions, and I also tender my thanks to Sir David Prain, C.M.G., the Director of the Royal Botanic Gardens, Kew, for allowing me to carry out the present investigation in the Jodrell Laboratory. I am also deeply indebted to Mr. L. A. Boodle, the Keeper of the Jodrell Laboratory, for his kindly criticism and valuable help.

¹ A berry of no particular shape is the derivational meaning of the word.

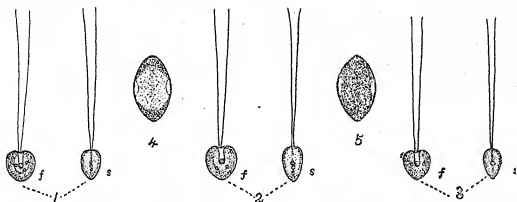
Scourfieldia cordiformis, a New Chlamydomonad.

BY

H. TAKEDA, D.I.C.

With five Figures in the Text.

A MINUTE unicellular Alga was recently discovered by the writer among some material of another Alga, kindly handed to him by Professor F. E. Fritsch, who had collected it last May in a *Sphagnum* marsh at Keston, Kent, and since then had kept it in the laboratory culture. The organism has proved to be a new species of *Scourfieldia*,¹ a very interesting genus of Chlamydomonadeae. The new Alga is very similar in many respects to the described species *S. complanata*, G. S. West,² but differs chiefly by the peculiar type of compression shown by an individual when viewed from the side. *S. complanata* is narrowly oblong in the side view, having both sides practically parallel to each other, while the side view of *S. cordiformis* is obovate (*s* in Figs. 1-3). In the front view the new



FIGS. 1-5. *Scourfieldia cordiformis*, Tak., sp. nov. Figs. 1-3, three individuals in front (*f*) and side (*s*) views. $\times 1,500$. Fig. 4, diagrammatic representation of anterior end view, showing nature of chloroplast. Fig. 5, ditto of posterior end view.

organism is heart-shaped (*f* in Figs. 1-3), hence the specific name *cordiformis* has been given. The chloroplast is constructed exactly in the same way as in *S. complanata*,³ being compressed bell-shaped with a comparatively large amount of colourless protoplasm within its central hollow. Another important character to be mentioned here is that the chloroplast, when viewed from the front, shows a median longitudinal slit reaching about half-way

¹ G. S. West, in Journ. Bot., 1912, p. 326, Fig. 3.

² l. c.

³ Cf. West, l. c., Fig. 1, F.

from apex towards the base (*f* in Figs. 1-3). The diagrammatic representations of the end views (Figs. 4, 5) may elucidate this peculiar feature more clearly. The chloroplast is, as in the case of *S. complanata*, absolutely homogeneous, and contains neither stigma nor pyrenoid. There are, however, apparently imbedded in the colourless protoplasm and in the proximity of the nucleus, two, or sometimes three, minute, highly refractive bodies of unknown nature. These bodies can readily be seen also in the side view of the organism (*s* in Figs. 1-3). The writer has not succeeded in detecting any contractile vacuoles.

Two flagella of equal length are attached at the anterior notch of the organism. They are very delicate, and reach a length of nearly four times that of the body of the organism.

As to the movements of this Alga, the writer has not been so fortunate as to obtain any satisfactory observations. The individuals examined were either too sluggish or too active to enable him to study their mode of locomotion properly.

As a unique character of *S. complanata*, this organism is known to move normally backwards, i.e. with its flagella behind.¹ This is a very unusual type of movement among the Chlamydomonads. Two or three other examples, however, observed by the writer may be mentioned here. A species of *Carteria*,² which occurred in the same material, often swims backwards for a considerable distance, whereas its normal method of locomotion is towards the anterior end of the organism. *Chlorogonium euchlorum*, Ehrb., usually swims forwards, but it sometimes moves backwards for a short distance (equal to about half or the whole length of the body), just as in the case of many Cryptomonads. Amongst the Englelineae, a variety (new) of *Trachelomonas hexangulata*, Swirenko,³ moves sometimes forwards and sometimes backwards. Under the description of *Tr. ampullula*, Mr. G. I. Playfair states⁴ that the organism 'swims backwards, with the orifice and flagellum behind'.

Unfortunately, so far no stage of reproduction of this new Alga has been observed.

DIAGNOSIS.

Scourfieldia cordiformis, Tak., sp. nov. (Figs. 1-5). Cellulae vegetativae minutissimae, valde compressae, a fronte visae cordiformes, polo apicali leviter emarginato et flagellis binis aequalibus, quam cellula quad-

¹ West, l. c., p. 327.

² Species indeterminata; *C. multifili* (Fresen.) Dill similis, sed cellula minus rotundata, membrana exteriori firma pro genere crassa, membrana interiori (= pars membranae gelatinosa) saepe valde evoluta differt.

³ In Archiv für Hydrobiol. u. Planktonk., ix, p. 646, Taf. xx, Figs. 23-5 (1914) = *Tr. ampullula*, Playfair, in Proc. Linn. Soc., N.S.W., xl, pt. 1, no. 157, p. 16, Tab. ii, fig. 6 (1915).

⁴ l. c., p. 17.

ruplo longioribus praedito, a latere visae obovatae vel anguste obovatae, in polum posterius obtusatum sensim attenuatae. Chromatophora. singula, viridis, homogenca, subcampanulata, sed compressa, apice ad medium fissa, sine pyrenoide; stigma et forsan vacuolae contractiles carentes; nucleus minutus, centralis. Long. cell. 4-4.5 μ ; lat. cell. 3.5-4 μ , crass. 2.3-2.6 μ ; long. flagellorum usque ad 20 μ .

A *S. complanata*, G. S. West, specie unica adhuc cognita, praesertim cellula a latere visa obovata nec anguste oblonga dignoscitur.

Hab. in Sphagnis, Keston, Kent (F. E. Fritsch., 1915).

The writer wishes to tender thanks to Sir David Prain, C.M.G., the Director of the Royal Botanic Gardens, Kew, for facilities for carrying out the present examination in the Jodrell Laboratory. Thanks are also due to Mr. L. A. Boodle, the Keeper of the Jodrell Laboratory, for his kind help during the preparation of this paper.



On the Plant Communities of Farm Land.

BY

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INTRODUCTION.

IT is proposed in the present paper to give some account of observations made over a period of eight years on the weeds found under various farm crops, including land put down to grass for various short periods and fields that have 'run down' to more or less permanent grass without the addition of seeds, or with a very inadequate sowing. Most of the work was carried out on the Cotswolds (360'-700') and in Mid-Wales (350'-1200'); a few observations have also been made near Holsworthy, Devonshire, and near Greenhithe, Kent.

Method of Study. Endeavour has been made to study the weed flora in terms of whole communities and not as isolated species; and to supplement careful observations by a more accurate method of procedure. Two statistical methods have been employed throughout the whole period of the work.

(a) *Specific Frequency.* This method is used on arable land and occasionally on grass-land. It is practically identical to that employed by Rhaunkiaer, which has been explained by Smith (8) in his review of that author's 'Life Forms and Statistical Methods'. The method has been described by the present writer elsewhere (10 and 11); its essence is to record the species found on a large number of small unit-areas, without paying any attention to the abundance of individual species per each unit-area. A mesh 6" x 6" is used and dropped at chance on the ground; the names of all the species occurring in each reading are carefully noted. The area to be examined is traversed across two diagonals and from 50 to 200 readings taken. The results given are recorded against each species in terms of the number of its occurrences per 100 readings.

For purposes of publication it is, however, found more convenient to tabulate the frequencies on a scale of 10; species with frequencies below 1 are marked 0, if occasional; s, if scarce or solitary; and r, if of quite exceptional occurrence.

(b) *Percentage Frequency.* This method, originally devised by Armstrong (1), is advantageously employed upon grass-land. It has been explained elsewhere (11). Small unit-areas (turfs 6" x 6") are removed

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from the field to be investigated, taken to the laboratory, the species separated out, and the number of plants belonging to each species is then counted and the results given as a direct percentage.

Specific frequencies have been taken on over 75 fields and several hundred have been examined altogether.

When frequencies or percentage figures are not available the species have been classified according to the following grouping, which experience suggests corresponds to the frequencies given against each :

Dominant,	e.g. frequencies 7-9 (10 is very exceptional)
Abundant,	" " 3-6
Frequent,	" " 1-2
Occasional,	" " less than 1 and as before
Scarce or Solitary,	" " " " "
Rare,	" " " " "

Treatment of the Subject. It will be convenient to deal with the subject-matter under two distinct heads. Part I. *The Weeds of Arable Land*; that is to say, the communities found under root crops, cereals, and 'seeds'. By 'seeds' is here understood leys which are sown down with a mixture of grass and clover seeds for one year only, or if sown for a longer period, till the end of the first year. The above communities are under the direct control of man, and owing to the exigencies of the rotation are short-lived and consequently do not afford material for the study of the progressive stages in the colonization of bare land. Part II. *The Weeds of Grass-land*. This includes the communities found on long duration leys and on permanent grass, and affords material for the study of the progressive changes which occur in the colonization of land under the constant influence of grazing animals. For the sake of comparison two cases will also be given of the colonization of bare land from which stock have been withheld.

PART I.

THE WEEDS OF ARABLE LAND.

Under this heading it is proposed to make the following comparisons :

- I. The aggregate arable flora of the Cotswolds with that of Mid-Wales.
- II. The results from Mid-Wales and the Cotswolds with those obtained elsewhere.
- III. The arable flora of Mid-Wales above and below 800 feet.
- IV. The weed flora under the several crops in the rotation.

In order to facilitate these comparisons the frequencies found for a large number of species in both localities and in various places in the rotation are set out in the subjoined table (Table I).

TABLE I.

DISTRIBUTION OF THE CHIEF SPECIES IN MID-WALES AND THE COTSWOLDS

Chief Species.	1.	2.	3.	4.	5.	6.	7.	8.	9.
	Roots.			Cereals.			Seeds.		
	Cotswolds.	Mid-Wales, below 800'.	Mid-Wales, above 800'.	Cotswolds.	Mid-Wales, below 800'.	Mid-Wales, above 800'.	Cotswolds.	Mid-Wales, below 800'.	Mid-Wales, above 800'.
<i>Ranunculus repens</i>	2	1	0-6	1	3 (6)	3-9	0	0-2	0-4
<i>R. parviflorus</i>	0	1	—	1	—	—	—	—	—
<i>R. arvensis</i>	1	—	—	—	—	—	—	—	—
<i>Papaver Rhoeas</i>	0	—	—	2 (5)	—	—	—	—	—
<i>P. dubium</i>	—	—	—	—	1	—	—	—	—
<i>Fumaria officinalis</i>	S-1	1	—	3-6 (9)	—	—	1	—	—
<i>F. pallidiflora</i>	—	0 (2)	1	—	0-1	—	—	—	—
<i>Erophila verna</i>	1	—	—	(4)	1	—	—	—	—
<i>Brassica arvensis</i>	0-1	S (2)	0	2-4	0 (5)	1 (6)	—	—	—
<i>B. nigra</i>	0	—	—	1	—	—	—	—	—
<i>Capsella Bursa-pastoris</i>	2	0	0	1	—	—	1	—	—
<i>Thlaspi arvense</i>	0	—	—	0	—	—	—	—	—
<i>Viola tricolor</i> (agg.)	0-1	1	1	1	2	0	3	1	0-1 (3)
<i>Silene Cucubalus</i>	1-0	—	—	1-3	S	1	—	—	—
<i>Lycnis alba</i>	0	—	—	1-2	S	1	—	—	—
<i>L. Chitago</i>	1	—	—	0	1	—	—	—	—
<i>Stellaria media</i>	2 (4)	3-5	3-6 (9)	2-5	3	2	1	1	0-(5)
<i>Arenaria serpyllifolia</i>	0	S	—	0	1	—	0-3 (6)	1	—
<i>Stergula arvensis</i>	—	1	1-9	—	1-4	4-6	—	1	—
<i>Hypericum pulchrum</i>	—	—	—	—	—	S-0	—	—	—
<i>Geranium molle</i>	0	S	—	S	0	S-0	1 (4)	0 (3)	S-2
<i>G. dissectum</i>	S	1	—	S-0	0	S	0 (3)	0 (2)	S-2
<i>Erodium cicutarium</i>	—	—	—	—	—	—	(3)	—	—
<i>Trifolium minus</i>	—	—	—	—	—	—	—	0-3	1-4 (7)
<i>Ornithopus perpusillus</i>	—	—	—	—	—	S-0	—	—	—
<i>Vicia Cracca</i>	—	—	S	—	—	S	—	—	—
<i>Lotus corniculatus</i>	—	—	1	—	—	S	—	—	S-0
<i>Lathyrus pratensis</i>	—	—	1	—	—	S	—	—	1
<i>Potentilla erecta</i>	—	—	0	—	—	(6)	—	—	S
<i>Alchemilla arvensis</i>	1	S-0	1	S-2	0	1	1-3	2	S-1 (2)
<i>Scandix Pecten-Veneris</i>	1	—	—	0	—	—	—	—	—
<i>Aethusa Cynapium</i>	1	—	—	1	1	—	—	—	—
<i>Daucus Carota</i>	S	1	—	—	—	—	—	0	—
<i>Galium saxatile</i>	—	—	—	—	—	0-2	—	—	S
<i>G. Aparine</i>	2	1	—	1	1	—	0	1	—
<i>Sherardia arvensis</i>	S	0	S	—	0	—	3	1	S
<i>Scabiosa arvensis</i>	1	—	—	1-2	1	—	—	—	—
<i>S. succisa</i>	—	—	—	—	S	0 (3)	—	—	—
<i>Bellis perennis</i>	1	S	1	—	3	2-9	S	1	0-2 (3)
<i>Gnaphalium uliginosum</i>	—	—	3	—	—	—	—	—	—
<i>Achillea Ptarmica</i>	—	—	—	—	—	0	—	—	—
<i>Chrysanthemum segetum</i>	—	2	0-4 (7)	—	0-4	1-7	—	—	—
<i>Matricaria inodora</i>	1	0	S	—	S	1	—	1	—
<i>Senecio vulgaris</i>	1	1	0	0	0	1	1	—	—
<i>Cnicus lanceolatus</i>	—	—	—	—	—	0	0	0	—
<i>C. arvensis</i>	3	1	S	3	2	0	—	—	—
<i>Centauria nigra</i>	—	S	0	—	—	S-3	—	—	—
<i>C. Cyanus</i>	—	—	—	0	—	—	—	—	—
<i>Cichorium Intybus</i>	—	—	—	—	—	—	1	0	S
<i>Lapsana communis</i>	—	0	S	0	0	S	—	S-0	S

Chief Species.	1.	2.	3.	4.	5.	6.	7.	8.	9.
	Roots.			Cereals.			Seeds.		
	Cotswolds.	Mid-Wales, below 800'.	Mid-Wales, above 800'.	Cotswolds.	Mid-Wales, below 800'.	Mid-Wales, above 800'.	Cotswolds.	Mid-Wales, below 800'.	Mid-Wales, above 800'.
<i>Crepis virens</i>	0	1	2	—	—	1	0	1-2	1-0
<i>Hieracium umbellatum</i>	—	—	—	—	—	s-2	—	—	—
<i>H. boreale</i>	—	—	—	—	—	s-0	—	—	—
<i>Hypochaeris radicata</i>	—	r	0	—	s	1-4	s	2	0-2
<i>Taraxacum officinale</i>	r	s	0	1	s	0	2	s	0 (3)
<i>Sonchus oleraceus</i>	1	2	s	0	—	—	—	—	—
<i>S. arvensis</i>	s	0	0	1-2	0	s	—	—	—
<i>Legousia hybrida</i>	r	—	—	0-3 (4)	—	—	—	—	—
<i>Anagallis arvensis</i>	0	s	—	0	2 (5)	—	r	r	—
<i>Myosotis arvensis</i>	0	s (2)	r	1	0	r	3	0	r-s
<i>Lithospermum arvense</i>	—	—	—	0	—	—	0	—	—
<i>Convulvulus arvensis</i>	6	0	—	4 (7)	0	—	r	—	—
<i>Veronica hederifolia</i>	2	s	—	7 (10)	s	—	r	—	—
<i>V. agrestis</i>	s	1 (4)	r	s	2	1-0	0	0	0-2
<i>V. polifolia</i>	—	0	0	—	0	0	—	—	—
<i>V. Tournefortii</i>	1	—	—	1	—	—	—	—	—
<i>V. arvensis</i>	r	s	—	s	0	—	s (2)	0	—
<i>V. serpyllifolia</i>	s	s	—	0	r	—	0-1	r-s	—
<i>Bartsia Odontites</i>	—	—	—	s	—	—	s	r	—
<i>Mentha arvensis</i>	—	0	1	—	1	0-2	—	s-0	s
<i>Clinopodium vulgare</i>	s	—	—	1	—	—	—	—	—
<i>Calamintha officinalis</i>	—	3	0-2	—	0	0	—	s	s-0
<i>Prunella vulgaris</i>	s	0	0	s	1	1-2	1	2-6	0-5
<i>Stachys arvensis</i>	—	1-2	—	—	1-2	r	—	—	—
<i>Galeopsis Tetrahit</i>	—	1	2-4	—	0	1-2	—	—	—
<i>G. versicolor</i>	—	—	0	—	—	s	—	—	—
<i>Lamium amplexicaule</i>	2	r	—	0 (6)	s	—	r	—	—
<i>L. purpureum</i>	2	—	—	s	s	—	r	—	—
<i>Plantago major</i>	s	s	s	s	s	s	r-r	r	—
<i>P. media</i>	—	—	—	0	—	—	s	—	—
<i>P. lanceolata</i>	r	s	1	s	0	1	1	1-5	7
<i>Scleranthus annuus</i>	—	—	—	—	0	0 (5)	—	—	—
<i>Chenopodium album</i>	3	2	s	2	s	—	—	—	—
<i>Atriplex patula</i>	2	2	0	0	0	—	—	—	—
<i>Polygonum Convulvulus</i>	2	1	0-1	2-5	0	s	—	—	—
<i>P. aviculare</i> (agg.)	3	1	0	3	1	0	r	r-s	—
<i>P. Persicaria</i>	3	4	1-0	0	0	1-4	r	r	s-0
<i>Rumex Acetosella</i>	—	2	3	—	3	6-9	—	0-2	0-4
<i>Euphorbia Helioscopia</i>	2	s	0	0	0	s	r	—	—
<i>E. exigua</i>	0	r	—	—	—	—	—	—	—
<i>Urtica urens</i>	r	—	—	—	—	r	—	—	—
<i>Allium vineale</i>	—	—	—	s	—	—	—	—	—
<i>Lunula erecta</i>	—	—	—	—	—	0-2	—	—	r-s
<i>Alopecurus myosuroides</i>	—	—	—	0	—	—	—	—	—
<i>Agrostis</i> spp.	1	1-7	3	s	1-6	2-8	0	1-3	0-2
<i>Aira caryophylllea</i>	—	—	—	—	r-s	0	—	—	r
<i>A. praecox</i>	—	—	—	—	r-s	0	—	—	r
<i>Holcus lanatus</i>	s	0-3	1	0	0	3	s	2	0 (3)
<i>Avena tirigosa</i>	—	—	0-3	—	—	0-3	—	—	—
<i>Arrhenatherum tuberosum</i>	—	0	1-4	—	s	1-2	—	—	—
<i>Poa trivialis</i>	—	—	—	0-2	—	—	—	—	—
<i>Festuca ovina</i>	—	—	—	—	—	1-2	—	—	—
<i>Bromus hordeaceus</i>	r	—	—	0	—	—	0	0	0
<i>Agropyron repens</i>	2	s	—	2	s	—	r	—	—

For some species the range of frequency is given (thus e.g. 4-8); in most cases the average frequency only is given (e.g. 5, or 8); in some cases as well as the average frequency an exceptionally high frequency is given (e.g. 3 the normal and (8) the exceptional figure).

I. Comparison of the Flora of the Cotswolds with that of Mid-Wales.

The soils of the two districts are very different; that of the Cotswolds is derived from the Great Oolite and is a highly calcareous loam.

The soils investigated in Mid-Wales are derived from Ordovician Shales and give rise to a thin, very stony soil, which in wet weather is very sticky (due to a high percentage of fine silt) and in dry weather tends to cake badly and form pan; under favourable conditions, however, it works into a friable loam.

Below (Table II) is given a typical chemical and mechanical analysis of each soil. For the Mid-Wales figures I am indebted to my colleague Mr. Jones Griffiths; the Cotswold results are quoted from Kershaw's (5) analyses.

TABLE II.

SYNOPSIS OF MECHANICAL AND CHEMICAL ANALYSES OF COTSWOLDS AND MID-WALES SOILS.

MECHANICAL ANALYSES.

	<i>Mid-Wales.</i>	<i>Cotswolds.</i>
Fine Gravel	20.3	1.5
Coarse Sand	13.5	1.6
Fine Sand	8.4	5.6
Coarse Silt	12.6	13.4
Fine Silt	20.8	15.3
Clay	7.8	13.8

CHEMICAL COMPOSITION.

	<i>Mid-Wales.</i>	<i>Cotswolds.</i>
Nitrogen	0.39	0.38
Lime (CaO)	0.15	11.27
Phosphoric Acid (P ₂ O ₅)	0.008	0.24
Potash (K ₂ O)	0.86	0.41
Available Phosphoric Acid	0.002	0.007
Available Potash	0.04	0.011

The above figures show the Mid-Wales soil to be very deficient in lime and phosphoric acid, the Cotswold soil to be very rich in lime and relatively rich in phosphoric acid. Considering the marked difference in the two soils the number of species which occur in the one district and not in the other are comparatively few. The following are, however, characteristic:

MID-WALES.

Spergula arvensis.
Ornithopus perpusillus.
Chrysanthemum segetum.
Stachys arvensis.
Galeopsis versicolor.
Scleranthus annuus.
Rumex Acetosella.

COTSWOLDS.

Ranunculus arvensis.
*Thlaspi arvense.**
Papaver Rhoeas.
*Lychnis Githago.**
*Erodium cicutarium.**
*Scandix Pecten-Veneris.**
Legousia hybrida.
Lithospermum arvense.
Clinopodium vulgare.
*Allium vineale.**

* These species have been noted by Salter (?) as occurring in Cardiganshire, but have not been seen on the arable lands investigated.

A number of species are far more abundant on the farm land of one district than the other. The following are good examples :

MID-WALES.

Ranunculus repens.
Calamintha officinalis.
Galeopsis Tetrahit.
Polygonum Persicaria.
Arrhenatherum tuberosum.

COTSWOLDS.

Silene Cucubalus.
Erophila verna.
Brassica nigra.
Geranium spp.
Aethusa Cynapium.
Scabiosa arvensis.
Lamium amplexicaule.
L. purpureum.
Convolvulus arvensis.
Alopecurus myosuroides.
Agropyron repens.

It is interesting in this connexion to note the behaviour of certain closely allied plants; the following are *the* species of three common genera for the two districts :

MID-WALES.

Fumaria pallidiflora.
Veronica agrestis.
Plantago lanceolata.

COTSWOLDS.

F. officinalis.
Veronica hederæfolia.
V. Tournefortii.
P. media.

These results will be further alluded to in the next section.

II. Comparison of Results obtained on the Cotswolds and in Mid-Wales with those recorded elsewhere.

The weeds of arable land have in the past been studied chiefly as individuals and not as communities; as long ago as 1845 Bravender (2) published an interesting paper on these lines, and this was followed in 1855 by a more exhaustive article by Buckman (4). Recently, however, Brenchley has investigated the weed *communities* of arable land on the following chief geological formations, Greensand, Chalk, Gault, and Boulder Clay, and has tabulated the results obtained in three important papers.¹ It will be of interest here to contrast the behaviour of some of the chief species found in the two districts under consideration with the relationships observed for the same species elsewhere, both by Brenchley and the present writer.

A. Cotswolds. It might be expected that the highly calcareous loams over Oolite on the Cotswolds would carry a very similar weed flora to the soils over Chalk; that the flora differs in many essentials will, however, be seen from the following comparison.

The following species are all common on the Cotswolds; but concerning each Brenchley in her most recent paper (Norfolk, 3 c) remarks as follows:

Ranunculus repens.* 'Distributed on all soils, but seldom seen on chalk.'

Erodium cicutarium. 'Distributed on sand and very light soils.'

Alchemilla arvensis. 'Characteristic of light and sandy soils, very rare on chalk.'

Scandix Pecten-Veneris.* 'Found on all soils, except chalk, though seen occasionally on chalky loam.'

Myosotis arvensis.* 'Chiefly on sand and loam, rare on chalk.'

Bartsia Odontites.* 'Chiefly associated with loam, never seen on chalk.'

Veronica hederaefolia. 'Associated with sand and light sandy loams, absent from clay and chalk. Once dominant on loam.'

Polygonum Convolvulus. 'Seen on chalky loam, but never on chalk.'

* These four species Brenchley finds in the Wiltshire and Bath districts are characteristic of Chalk.

Thus it appears that all the above species, although in many districts essentially non-Chalk plants, may be associated with highly calcareous as well as with more normal loams. *Veronica hederaefolia* is particularly interesting, for on the Cotswolds it is one of the commonest weeds, where it generally has a frequency corresponding to a dominant position, yet 'it is absent from chalk and only once dominant on loam'.

¹ Brenchley, W. E.: (3 a), (3 b), (3 c).

A number of species common on Chalk are also typical Cotswold weeds; some, however, appear to have rather different frequencies over Oolite than on other calcareous soils. The distribution of the following plants on the Cotswolds may be contrasted with their distributions recorded elsewhere by Brenchley; the remarks in inverted commas after each species are quoted from this author.

Papaver Rhoeas. 'Often dominant', true of the Cotswolds.

Fumaria officinalis. 'Occasionally dominant', frequently dominant on the Cotswolds.

Lychnis Githago. 'Scarce in distribution', occasionally plentiful on the Cotswolds.

Silene Cucubalus. 'Twice dominant on sand, usually distributed or occasional', often abundant on Cotswolds.

Legousia hybrida. 'Never dominant, often scarce', frequently very abundant on Cotswolds.

Neither *Linaria vulgaris* nor *Cichorium Intybus* are plentiful on the Cotswolds.

It would thus appear that the weed communities of the Cotswolds, especially when the frequencies of the chief contributing species are taken into consideration, are decidedly characteristic, and that they differ both from the communities found on ordinary loams and from those found over Chalk.

B. Mid-Wales. These soils are acid in reaction and consequently decidedly 'sour'; they are by no means sands; they are intermediate between loam and clay, and are thus 'sour' soils of a heavier nature than those investigated by Brenchley.¹ Heavy 'sour' clays have been studied by the present writer near Holsworthy, Devon, and peats have come under observation in Mid-Wales, while the flora on sands have been examined near Greenhithe, Kent. It is possible, therefore, to contrast the communities found on the various grades of 'sour' soil.

Below (Table III) is given the range of frequencies for the chief species found on four grades of 'sour' soils: (1) Peat (Mid-Wales); (2) Non-calcareous clay (Devon); (3) Non-calcareous stiff loam (Mid-Wales); (4) Sand (deduced from Brenchley's papers and the present writer's personal observations in Kent).

¹ See Brenchley, (3 c), p. 149.

TABLE III.

TO COMPARE THE WEED COMMUNITIES FOUND ON FOUR GRADES OF 'sour' SOIL.

Chief Species.	1. Peat.	2. Non- Calcareous Clay.	3. Non- Calcareous sticky loam.	4. Sand.
<i>Ranunculus repens</i>	a-d	a	f-a	o-f
<i>Papaver Rhoeas</i>	—	—	—	a-d
<i>Erophila verna</i>	—	—	r	o-f
<i>Spergula arvensis</i>	a-d	f-a	a-d	a-d
<i>Geranium molle</i>	—	—	r-f	f-a
<i>G. dissectum</i>	r	o-f	r-f	o-f
<i>Potentilla Anserina</i>	—	o-f	f-a	r
<i>Scabiosa succisa</i> *	a-d	f-a	r	f-a
<i>Gnaphalium uliginosum</i>	r	f	o-f	—
<i>Achillea Ptarmica</i> *	o-f	f	r	d
<i>Chrysanthemum segetum</i>	o-f	r	a-d	—
<i>Centaurea nigra</i> *	o-f	f-a	o-f	r
<i>Lyophilis arvensis</i>	—	—	—	o
<i>Echium vulgare</i>	—	—	—	o
<i>Veronica hederaefolia</i>	—	—	r	o-f
<i>Mentha arvensis</i>	f-a	f-d	f	r-o
<i>Prunella vulgaris</i>	f-a	f-a	f-a	o
<i>Stachys palustris</i>	—	f-a	—	—
<i>S. arvensis</i>	—	f	f	—
<i>Galeopsis Tetrahit</i>	f	f	f-a	—
<i>G. versicolor</i>	o-f	—	f	—
<i>Scleranthus annuus</i>	o	a	f	a-d
<i>Polygonum Persicaria</i>	a-d	f	f-a	f-a
<i>Rumex Acetosella</i>	d	a	a	a-d
<i>Euphorbia exigua</i>	—	o	o	r
<i>Aira caryophyllaea</i>	s-o	o	o	f-a
<i>A. praecox</i>	f	o	s	f
<i>Bromus hordeaceus</i>	—	r	s	f

* These species, although most common on marshy pastures, have also been found on arable land.

The behaviour of the following species is of particular interest. *Spergula arvensis* is seen to be by no means essentially a sand plant; it may be abundant on all 'sour' soils and dominant on clay and sand alike. *Chrysanthemum segetum* is also distributed over 'sour' soils generally; although rare on clays, it may be dominant on sticky loams and frequent on peat. *Rumex Acetosella* is most abundant on peat and sand. *Potentilla Anserina* and *Gnaphalium uliginosum* are both favoured by winter puddling; the latter plant has been commonly found about gates, and near Greenhithe was the only plant on the floor of an orchard, on sand on which pigs had been kept all the winter.

Scleranthus annuus has also been found on all grades of 'sour' soil, even attaining to an abundant position on clay. *Ranunculus repens* may completely overrun peat soils.

It appears from a general consideration of the above table that the communities judged as a whole are more symptomatic of soil type than are the presence or absence of relatively few index plants; and it must be

urged that, although *Spergula arvensis* and *Chrysanthemum segetum* are certain indications of 'sourness', they are not confined to sands alone. It will be noted that less than 25 per cent. of these 'sour' soil plants are calcifuge species, suggesting that circumstances influencing the available water of the habitat, aeration, and other environmental factors exert as much influence as the absence of lime as such.

It is of interest to observe that the Mid-Wales soils, although ranging from clay to stiff loam, do not include *Ranunculus arvensis* in their weed communities, and that such plants as *Stachys palustris*, *Euphorbia exigua*, *Galium Aparine*, *Potentilla reptans*, *Agropyron repens*, and *Papaver Argemone* are not at all generally distributed. *Euphorbia Helioscopia*, on the other hand, is probably as frequent on these soils as on those of a more calcareous nature.

III. The Communities of Mid-Wales above and below 800'.

Smith and Moss (9) and Moss (6) have pointed out that the limit of Wheat cultivation corresponds roughly with the limits of a number of weeds. Wheat is not grown on an extensive scale in the area now under consideration, but a number of farmers at altitudes below about 800' grow a small breadth for home consumption. Oats are grown up to about 1,400', and Barley is often to be seen considerably above 1,000'. Moss (6) has shown that the altitudinal limit of wheat cultivation is different on the various soils of the Peak district, but the average range is from about 500' to 900'. In this district the general method of husbandry is usually somewhat different above about 800' than below it, so that the 800' contour is a useful line of demarcation for purposes of comparison. The chief differences in the farming at high altitudes, apart from the absence of Wheat, are that the Brown Oat (*Avena strigosa*) is more extensively grown; Mangolds are only grown to a slight extent; and the grass is left down for a longer period than is the case at lower elevations. Furthermore the arable farming is usually negligent; the hoe is frequently inactive; and the grass and clover seeds used are poor in quality, full of impurities, and inadequate. The effect of these primitive methods of husbandry on the weed flora will be shown to be considerable.¹

Moss (6) has listed some forty odd species for the area studied in the Peak district as not occurring on both the Wheat and no-Wheat zone, practically all of these being absent from the higher elevations.

A comparison of columns 3, 6, 9 (no-Wheat zone, i.e. above 800') with 2, 5, 8 (Wheat zone, i.e. below 800') in Table I shows the following differences for Mid-Wales:

¹ It is perhaps most marked in the case of the grass-land. See Part II.

- (a) Species only occurring at the Lower Elevations; but which even then are in most cases rare or occasional.

<i>Europhila verna.</i>	<i>Veronica hederacfolia.</i>
<i>Papaver dubium.</i>	<i>V. arvensis.</i>
<i>Scabiosa arvensis.</i>	<i>Lamium amplexicaule.</i>
<i>Aethusa Cynapium.</i>	<i>L. purpureum.</i>
<i>Convolvulus arvensis.</i>	<i>Euphorbia exigua.</i>

- (b) Species rare or exceptional at the Higher Elevations.

<i>Fumaria pallidiflora.</i>	<i>Arenaria serpyllifolia.</i>
<i>Silene Cucubalus.*</i>	<i>Bromus hordeaceus.</i>
<i>Lychnis alba.*</i>	

- (c) Species decidedly more abundant at the Lower Elevations.

<i>Alchemilla arvensis.</i>	<i>Lapsana communis.*</i>
<i>Galium Aparine.*</i>	<i>Sonchus oleraceus.</i>
<i>Sherardia arvensis.*</i>	<i>S. arvensis.</i>
<i>Matricaria inodora.*</i>	<i>Anagallis arvensis.</i>
	<i>Myosotis arvensis.</i>
	<i>Stachys arvensis.</i>
	<i>Chenopodium album.*</i>

- (d) Species frequently more abundant at the Higher Elevations.

<i>Ranunculus repens.</i>	<i>Hieracium umbellatum.†</i>
<i>Spergula arvensis.*</i>	<i>H. boreale.†</i>
<i>Hypericum pulchrum.†</i>	<i>Galeopsis Tetrahit.</i>
<i>Vicia Cracca.†</i>	<i>G. versicolor.</i>
<i>Lotus corniculatus.†</i>	<i>Rumex Acetosella.*</i>
<i>Lathyrus pratensis.†</i>	<i>Luzula erecta.†</i>
<i>Potentilla erecta.†</i>	<i>Arrhenatherum tuberosum.</i>
<i>Chrysanthemum segetum.*</i>	<i>Festuca ovina.†</i>

- (e) Species which Moss has noted as absent on the no-Wheat zone in the Peak district; but which in Mid-Wales are freely distributed above 800'.

<i>Geranium molle.*</i>	These species do not, however, attain to their full luxuriance at the higher elevations.
<i>G. dissectum.*</i>	
<i>Veronica agrestis.</i>	
<i>Mentha arvensis.</i>	

* These species are all more or less common impurities in clover seeds. See Stapledon, R. G.

(12).

† These species are normally weeds of grass-land. See p. 172.

It thus appears that in Mid-Wales there are only about twenty-five species which belong more essentially to the Wheat than to the no-Wheat zone, and that the majority of the abundantly occurring weeds are common to both zones. A number of the species which occur to only a slight extent at the higher elevations undoubtedly owe their origin to being introduced with the seed mixtures. Commonly occurring impurities have been marked with an asterisk in the above lists; many of these, although normally weeds of young leys, may not appear till the land has been again ploughed, when they are manifest as isolated weeds in the corn, but being unsuited to high elevations remain small and stunted.¹

The reason for the greater abundance of certain species at the higher than the lower elevations is probably not climatic.

Thus the more frequent dominance of *Spergula arvensis*, *Chrysanthemum segetum*, *Ranunculus repens*, and *Rumex Acetosella* is simply due to absence of the hoe; whilst neglect in the use of the horse-hoe would account for the prominent position of *Arrhenatherum tuberosum*. Similarly *Galeopsis Tetrahit*² and *G. versicolor*, normally waste-place plants, are able to flourish amidst neglected root crops. The presence of such plants as *Hypericum pulchrum*, *Vicia Cracca*, *Potentilla erecta*, and others marked with a dagger under section (d) is explained as follows:

These plants do not occur to any extent under roots, but are often common under Oats. Oats always follow grass in the rotation at high elevations; in many cases, however, the grass may have been down for eight to ten years, when it will have become very inferior, full of weeds, and of an indigenous character; many of the indigenous plants are able to survive the process of ploughing and again appear under the first Oat crop, although not ordinarily speaking arable land weeds. *Avena strigosa*, grown as a cereal, occurs as an abundant weed in the following root crop.

IV. The Communities under the several Crops in the Rotation.

The weed flora found under different crops is partly influenced by the manner of growth and partly by the tillage operations connected with the husbandry of the particular crop. Under good farming arable land should be free from a number of familiar perennials, while a well-tended root crop should at all times be associated with but a meagre weed community; under indifferent farming, however, 'roots' present very favourable opportunities for the spread of weeds, namely, a comparatively rich soil and abundance of bare ground.

There can be no doubt also that arable land communities are affected throughout the whole rotation by impurities sown with the grasses and clovers; this is particularly noticeable in districts where high farming

¹ e.g. *Galium Aparine*, *Myosotis arvensis*, and *Silene Cucubalus*.

² Seeds of this plant are frequently introduced with Oats,

is not the rule. The influence of impurities on the communities at high elevations in Mid-Wales¹ has already been referred to, and probably accounts for the sporadic appearance of weeds such as *Silene Cucubalus*, *Caucalis nodosa*, *Matricaria inodora*, *Lapsana communis*, and *Galium Aparine* in the arable land. It is now generally accepted that seeds of a great number of species can lie dormant in the soil for considerable periods; and it has been shown elsewhere² that poor grass and clover samples contain the seeds of a number of weeds common to root and cereal crops, which on favourable soils and under the influence of tillage operations probably manifest themselves as considerable nuisances, and on unfavourable soils may give rise to sporadic appearances of unusual weeds.

The communities studied both on the Cotswolds and in Mid-Wales appear to be somewhat different under roots, cereals, Vetches, and 'seeds', as is shown by the following brief synopsis.

(a) *Cereals and Roots.*

The communities under cereals in particular have been elsewhere (10) shown to exhibit a regular seasonal change. The flora found late in June and during July (when the straw is reaching its maximum height) includes relatively fewer ground annuals than is the case earlier in the spring, and more tall, straggling, and climbing plants such as *Sonchus arvensis*, *Polygonum Convolvulus*, and *Convolvulus arvensis*, and also plants the seed of which germinates later in the season, e.g. *Aethusa Cynapium*.

The flora on the stubble after the corn has been harvested is often an exceedingly rich one, and is more or less a replica of that occurring in the seedling corn early in the spring. For the purpose of the present comparisons the communities found under cereals from March till the middle of June are considered as representative.

Certain species appear to be rare under roots; on the Cotswolds this is true of

Ranunculus arvensis.

Erophila verna.

Lychnis Githago.

Bartsia Odontites.

Plantago media.

Poa trivialis.

Under poor farming on some of the Mid-Wales soils, certain species, which are not normally common in the root crop elsewhere, may be fairly plentiful:

Alchemilla arvensis.

Bellis perennis.

Lapsana communis.

Matricaria inodora.

Plantago lanceolata.

Holcus lanatus.

¹ Will be discussed in greater detail when dealing with grass-land.

² See Stapledon, R. G. (12).

A few species are, as Brenchley has observed, practically confined to cereals; on the Cotswolds this is true of

Lapsana communis.

Poa trivialis.

Plantago media.*

* Becomes very abundant on leys that have been down for some years.

In Mid-Wales this distinction does not hold, but at high elevations certain indigenous grass-land plants invade the corn crop only.¹

The above distinctions in terms of individual species are slight; if, however, the frequencies of some of the more abundantly occurring species are compared, it will be seen that the differences in the communities are more marked.

The following species have higher frequencies under cereals than roots:²

COTSWOLDS.

Papaver Rhoeas.

Fumaria officinalis.

Brassica arvensis.

Silene Cucubalus.

Lychnis alba.

L. Githago.

Scabiosa arvensis.

Taraxacum officinale.

Legousia hybrida.

Lithospermum arvense.

Veronica hederacfolia.

Polygonum Convolvulus.

MID-WALES (below 800').

Ranunculus repens.

Brassica arvensis.

Spergula arvensis.

Chrysanthemum segetum.

Rumex Acetosella.

The following species tend to have higher frequencies under roots than cereals:

COTSWOLDS.

Capsella Bursa-pastoris.

Senecio vulgaris.

Lamium purpureum.

Polygonum Persicaria.

Atriplex patula.

Euphorbia Helioscopia.

MID-WALES (below 800').

Sonchus oleraceus.

Calamintha officinalis.

Polygonum Persicaria.

Atriplex patula.

It is also noteworthy that a number of species, although not necessarily

¹ See p. 171, section (d), species marked with a dagger.

² In districts where farmers sow cereals saved from their own stacks, the following weed seeds are frequent impurities: *Lychnis Githago*, *Polygonum Persicaria*, *P. Convolvulus*, *Convolvulus arvensis*, and *Rumex* spp.

having higher frequencies under roots, are much more luxuriant on a well-manured root field than they are under cereals; examples are—

Capsella Bursa-pastoris.

Stellaria media.

Senecio vulgaris.

Sonchus oleraceus.

Anagallis arvensis.

Chenopodium album.

Atriplex patula.

Lamium purpureum.

At high elevations, or elsewhere where the farming is poor, differences between the root and cereal communities are not so well marked; and in this connexion it is interesting to note that a number of more or less typical corn weeds are usually either quite sporadic in roots or absolutely overrun the crop. They can be checked by the free use of the hoe, but if this is neglected they become even more abundant than under corn. Thus the normally greater abundance of *Papaver Rhoeas*, *Brassica arvensis*, *Spergula arvensis*, and *Chrysanthemum segetum* under corn than roots is due almost entirely to immunity from mechanical disturbance.

(b) Vetches.

The communities under this crop are usually meagre, for it is essentially a 'smothering' crop; consequently the number of small ground annuals is inconsiderable. The presence of larger perennials will depend on the state of cleanliness of the ground. On the Cotswolds the following plants were usually found able to compete favourably with Vetches—*Ranunculus arvensis*, *Galium Aparine*, *Convolvulus arvensis*, and *Polygonum Convolvulus*; and in dry seasons (e.g. 1911) certain tall plants introduced with the seeds are sometimes successful, e.g. *Brassica alba*, *Lychnis Githago*, *Saponaria Vaccaria*, *Linum usitatissimum*, and *Cannabis sativa*.*

* Introduced with German and Russian samples of seed.

(c) 'Seeds.'

The weed communities under seeds are more characteristic than those associated with other crops. Weeds under 'seeds' have to contend with the strong inter-specific competition set up by the growth of several million seedlings of plants having decidedly gregarious characteristics.¹ These plants form a considerable tangle on the ground by the first autumn after sowing. It follows that the weed flora will be considerably affected by the degree of excellence with which the seeds 'take.' This will depend

¹ e.g. from about 4.5 to 5.5 million for short leys and 18 million for long duration pastures. Observations on the Cotswolds and in Mid-Wales have shown that 1 million weed plants to the acre is an exceptionally high figure under cereals and roots. Oats give about 3 million and Barley 2 million sown seedlings to the acre, so that the aggregate competition under Oats is between something under 4 million and Barley something under 3 million seedlings per acre, and there is also much more space between the cereal seedlings than between those of the grasses and clovers.

chiefly on (a) the season, (b) the soil, (c) the appropriateness of the seed mixture used.¹ In dry seasons and on poor soils the 'take' is likely to be poor, in consequence of which the weed flora will include a number of species not usually associated with good leys. The following synopsis may be given of the weeds met with under 'seeds':

- (a) Those which do not occur elsewhere in the rotation.

<i>Cuscuta racemosum</i> *	} (Mid-Wales).
<i>Trifolium agrarium</i> *	
<i>Cichorium Intybus</i> *	
<i>Cnicus lanceolatus</i> * (Mid-Wales and Cotswolds).	

- (b) Those which under good 'takes' are normally found to have high frequencies—frequencies for many of the species higher than found elsewhere in the rotation.

<i>Ranunculus repens</i> *	<i>Bellis perennis</i> .
<i>Viola tricolor</i> (agg.).	<i>Cnicus lanceolatus</i> *
<i>Arenaria serpyllifolia</i> .	<i>Hypochoeris radicata</i> *
<i>Cerastium</i> spp. *	<i>Taraxacum officinale</i> * ²
<i>Geranium molle</i> *	<i>Myosotis arvensis</i> *
<i>G. dissectum</i> *	<i>Veronica serpyllifolia</i> .
<i>Erodium cicutarium</i> .	<i>Prunella vulgaris</i> *
<i>Alchemilla arvensis</i> .	<i>Plantago lanceolata</i> *
<i>Trifolium minus</i> *	

- (c) On poor soils and under moderate 'takes' the following are frequent:

Lotus uliginosus (Mid-Wales on wet soils).
Chrysanthemum leucanthemum * (Mid-Wales and Cotswolds).
Veronica agrestis (Mid-Wales).
Rumex Acetosella * (Mid-Wales, especially on peat).
Holcus lanatus * (Mid-Wales and Cotswolds).
Bromus hordeaceus (Cotswolds).
Agrostis spp. (Mid-Wales).

- (d) In dry seasons and wherever the 'take' may have been for any reason very poor the following are prone to occur:

<i>Capsella Bursa-pastoris</i> .	<i>Polygonum Persicaria</i> *
<i>Fumaria officinalis</i> (Cotswolds).	<i>P. aviculare</i> .
<i>Stellaria media</i> *	<i>Euphorbia Helioscopia</i> .
<i>Galium Aparina</i> *	<i>Agropyron repens</i> .
<i>Veronica hederacfolia</i> (Cotswolds).	

¹ This point will be dealt with at length under grass-land. See Part II.

² This plant has been noted by Brencley to be absent or rare under 'seeds'; this is by no means the case in Mid-Wales.

- (e) Species, although abundant in a district which, are quite exceptional under even the poorest 'take'.

Spergula arvensis.*

Legousia hybrida.

Sonchus arvensis.

Chenopodium album.*

Chrysanthemum segetum.*

* The seeds of species thus marked in the above lists are all more or less plentiful in poor seed mixtures.

It appears from a consideration of the above lists that except for *Cnicus lanceolatus*, which is perhaps a typical weed of both young leys and older grass-land, the only plants met with under 'seeds' that do not occur elsewhere in the rotation are certain exotics¹ introduced with the clover seeds and which can grow under grass-land conditions.

If the plants mentioned under headings (b) and (c) are regarded as being characteristic weeds in 'seeds' on soils that suit them, it would seem that the following growth forms are well adapted to compete with the sown turf-forming and gregarious species.

A. Plants which produce Seedlings capable of attaching themselves closely to the ground.

(a) Annuals.

The most successful annuals are those which either in the first or second generation form little cushions on the ground (they appear thus in the late autumn); these autumnal plants do not, however, flower till the following spring—that is to say, they are, under the conditions obtaining, hibernal annuals. Good examples are: *Viola tricolor* (agg.) (often from second generation), *Alchemilla arvensis* (usually first generation), *Trifolium minus* (first or second generation), and *Myosotis arvensis* (usually first generation).

Even more successful are those definitely hibernal annuals which form strong rosettes on the ground during the first autumn, e.g. *Geranium molle* and *G. dissectum*, and *Erodium cicutarium* (this plant often becomes a biennial or even a short-lived perennial).

(b) Biennials and Perennials.

The most successful are plants which during the first autumn produce considerable cushions, mats, or rosettes close on the ground, and subsequently develop a spreading or creeping manner of growth, or send up comparatively long flowering stems. Examples of the first type are *Ranunculus repens*, *Bellis perennis*, *Chrysanthemum leucanthemum*, *Veronica serpyllifolia* (shortly creeping), *Prunella vulgaris*, and *Rumex Acetosella*; and of the second, *Cnicus lanceolatus*, *Hypochaeris radicata*, *Taraxacum officinale*,

¹ Exotic to the particular district.

and *Plantago lanceolata*. It will be shown (Part II) that the majority of these plants are capable of gaining considerably on grass-land as the years go on.

B. Plants the Seedlings of which do not attach themselves unusually closely to the ground.

(a) *Gramineae*.

On poor soils *Holcus lanatus* and *Agrostis* spp. in Mid-Wales and *Bromus hordeaceus* on the Cotswolds may be fairly plentiful even in the first year of a ley, although they only become abundant as the rye grasses die off; thus these grasses can compete to some extent with the sown species.

(b) *Other Natural Orders*.

The commonest plants are *Arenaria serpyllifolia*. This annual is capable of spreading considerably over the ground, and under Sainfoin leys especially has a very gregarious habit. *Veronica agrestis* usually occurs on leys at high elevations in Mid-Wales; observations suggest that it may there produce a few flowers in the autumn, but none the less live over the winter and flower more freely during the following spring.

Some plants, although endowed with favourable growth forms (when judged by the above standards), are none the less rare or exceptional on leys. A good example is *Agropyron repens*, a tall-growing grass with an extensive system of stolons. This plant does not seem able to compete with other gregarious plants; possibly it requires more light and air than is available under grass-land conditions.

The plants which may be met with on poor leys and in dry seasons are for the most part ephemeral annuals which can thrive on bare patches, but the seedling plants of which, not having a cushion form of growth, are rapidly suppressed when the conditions are again favourable to the spread of the grasses and clovers. Colonies of these plants are frequent where the corn has been leysed and where, consequently, the seed 'take' has been bad—very successful species then being, *Stellaria media* in Mid-Wales, and *Veronica hederacfolia* on the Cotswolds.

In conclusion, it must be pointed out that a number of weed impurities are introduced with grass and clover seeds (especially in districts where the farming is poor), but if the 'take' is good only such as are capable of growing under 'seeds' will appear to any extent in the ley; exceptionally large amounts of *Geranium* spp. and other species marked with an asterisk in groups (b) and (c) are frequently to be attributed to this cause.¹

¹ A number of fields in Mid-Wales have been examined in the light of the impurities found in the samples of seeds sown.

CONCLUSION.

One object of the present paper has been to show that considerable advantages are to be gained by studying the weeds of arable land on a statistical basis, and in the light of the community as a whole. The application of this method immediately shows that, apart from anything else, species differ very much in their powers of colonization. Certain species, although they may be generally distributed over districts, are never numerically abundant; others are, however, capable of forming considerable carpets on the ground.

For instance, *Ranunculus repens*, *Rumex Acetosella*, *Spergula arvensis*, and *Veronica hederæfolia*, and a number of other plants under congenial surroundings may have frequencies as high as 9. *Euphorbia Helioscopia*, *Lapsana communis*, and other plants, although they may grow under equally congenial surroundings, seldom attain to frequencies as high as 2. Thus when contrasting the behaviour of species under different conditions, it is necessary to have in mind their inherent capabilities as colonizers; consequently the presence of such weeds as *Spergula arvensis*, *Ranunculus repens*, &c., in very small amount may, in certain cases, be just as or more significant than the complete absence of a species with a low habitual frequency.¹ It can be shown, furthermore, that a knowledge of the habitual frequencies of species makes it possible to gauge with some degree of accuracy their behaviour under unusual seasonal or other change. For instance, on the Cotswolds in the spring of 1912 (i.e. following the drought of 1911), a few species doubled and in some cases trebled their habitual frequencies, e.g. *Lamium amplexicaule* and *Arenaria serpyllifolia*, whilst *Veronica hederæfolia* everywhere attained to something approaching its maximum figure. Important, however, as it is to take frequencies into account, it is far more important to contrast whole communities, or at all events the chief contributing species of communities, rather than to interpret the influences of soil or of cropping in terms of the behaviour of certain 'index' plants; this has been emphasized in the body of the paper, and was well exemplified when considering the flora of 'sour' soils.

The results given would seem to show that the weed communities of arable land are (1) decidedly responsive to change in soil; (2) are different near the altitudinal limits of cultivation, to what they are on the same soils at lower elevations; this is, however, in part due to negligent husbandry; (3) that they are also influenced by the crop under which they grow, but that this is largely due to the husbandry associated with the various crops.

It has only been sought to compare the communities under Roots (including Swedes, Mangolds, and Potatoes), Cereals (including Wheat,

¹ The presence of species with high habitual frequencies in small amount only may be due to the activity of the hop—a source of error always to be guarded against.

Oats, and Barley), Vetches and 'Seeds'. It has been pointed out that under good farming the communities with roots are meagre, but that certain species are usually more luxuriant under roots than when associated with other crops. Under poor farming the communities met with in roots and cereals do not differ much from each other. 'Seeds' have been shown to favour characteristic communities, and something definite can be asserted as to the growth forms of the generally successful plants; the nature of the community is, however, considerably influenced by success or otherwise of the sown seeds. (4) In districts where inferior and unclean seeds mixtures are used, the communities not only under the seeds, but in subsequent crops in the rotation, may be influenced to a large extent by the added impurities.

THE FAUGAN,
LLANBADARN,
ABERYSTWYTH.

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Parallel Tests of Seeds by Germination and by Electrical Response.

(Preliminary Experiments.)

BY

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INTRODUCTION.

IN previous work it has been shown by Professor A. D. Waller¹ that there is a definite electrical response in the case of living seeds, which is no longer given when the seed is dead. The present experiments were undertaken with the view of further developing this electrical test, in regard to its possible commercial application. When the necessary experimental procedure has been settled, and the conditions studied more fully, the electrical response should furnish a more rapid and definite indication of the vitality of a set of seeds than the ordinary germination method. It would supply the practical man's demand for a trustworthy answer 'while you wait'.

METHOD OF EXPERIMENTS.

(i) *Germination*.—Germination was allowed to proceed in the usual way, the grains being placed between filter-paper moistened with a known quantity of distilled water, or with various food solutions, in suitable dishes and kept for a certain time (24 to 48 hours) at a known temperature. The electrical response was then recorded. Those having germinated at the end of the time were counted, and the percentage of the whole calculated. The average of a number of counts was taken as the *germination value*. 100 grains were found to be a convenient number to use, and the total germination value was recorded at the end of 6 to 10 days.

In these experiments the grains used were all of one species—*Hordeum vulgare*, the common barley—on account of the greater ease of manipulation of the larger grain. The observations would necessarily be extended to other seeds, where special devices might have to be adopted on account of small size, &c.

¹ Proc. Roy. Soc., vol. lxxviii, 1901, p. 79.

Different samples of barley were very kindly sent for the purpose by Mr. Stapleton of Aberystwyth.

(ii) *Electrical Method.*—The embryos were dissected from the grain, and connected to the circuit which Professor Waller describes in his paper on the vitality of seeds, in such a way that a current passing in the direction root \rightarrow stem causes a deflexion of the galvanometer from left to right. This direction is designated by the sign +, indicating a 'positive' response from *B* to *A*.

The circuit consists of:

- (1) A compensatory circuit.
- (2) Two galvanometers in series of greater and lesser sensitiveness respectively, the deflexions of which are thrown on to a specially constructed scale, which is then easily read.
- (3) An induction coil capable of giving single induction shocks of known strengths and direction.

By a suitable arrangement of plugs any of these branch circuits can be short circuited.

When the embryo is adjusted the galvanometer plug is opened, and any current in circuit due to the plant material noted. This is immediately balanced by throwing in, from the compensator, an equal current in the opposite direction to the accidental current. A standard for the electrical response is then obtained by ascertaining the deflexion of galvanometer caused by sending into the circuit 0.01 volt from the compensator. From this the magnitude of the response can afterwards be calculated. The apparatus is adjusted so that the galvanometer deflexion is at zero. Next a single-break induction shock is sent into the plant, the galvanometer circuit being closed at the time of shock, and opened directly after, so that only the response of the plant may be recorded. A series of four shocks is applied, and the corresponding responses noted, the order in which they are given being: (1) +1,000, (2) -1,000, (3) +10,000, (4) -10,000, in notation of a Berne coil supplied by two Leclanché cells. The response, if large, is read on the scale of the less sensitive galvanometer; if small, on that of the more sensitive one.

Any residual current aroused by an induction shock is balanced from the compensatory circuit before another is delivered.

At the end of the series the value of 0.01 volt is again recorded.

This furnishes the routine of the experiments carried out, an average of ten experiments being taken as a rule.

In Table I an example of ten experiments is given.

OBJECT OF EXPERIMENTS.

To determine how far there was a correspondence between the average germination value and the electrical response.

Series of experiments to record the two values were carried out at the same time—and for each experiment—under the same conditions.

The problem was attacked in several different ways:

(i) Using the same samples of grain giving the same average germination value.

Records were taken to ascertain if there was a fairly consistent response under approximately similar conditions of experiment. Here, of course, *exactly* consistent responses could only be looked for if great care was taken to ensure exactly similar conditions as regards temperature, moisture, electrodes, &c. Small variations in these conditions would probably produce corresponding variations in the electrical response, and these would affect the absolute magnitude of the average of responses.

(ii) Using the same sample of grain.

Experiments were carried out to determine whether variation in the conditions affecting the germination favourably or the reverse affect the electrical response in the same way. Two parallel determinations are carried out in this case.

(iii) Using different samples of grain giving different germination values.

These were compared under constant conditions as far as possible.

RESULTS OF EXPERIMENTS.

General.—The current of injury or accidental current is in the 'positive' direction—from root to stem—although the opposite was noted in the majority of cases.

The response to shocks is regularly in the 'positive' direction, but occasional alterations in the negative direction were observed. The significance of this alteration has yet to be determined. It may be an important detail.

The greatest response is noticed in the case of the first strong shock, the second strong shock giving just as regularly the smallest response—due, no doubt, to fatigue.

The actual magnitude of the response varies with other factors as already suggested, and would be affected by the temperature at which the experiment was carried out, and the atmospheric conditions at the time.

Further investigation would probably indicate more definitely the extent of the variation, if care was taken to record the temperature, pressure, &c., exactly.

(i) The general nature of the electrical response for the *same* sample of grain was quite consistent. Even the germination values vary slightly, and it was found that the number of seeds growing in 100 was not always identical in the different experiments.

TABLE I (May 4, 1915).

Hordeum vulgare. 27 hours' germination; distilled water; 85 % = germination value.

<i>Acc. C.</i>	$1/100.$	<i>R. x 2,000</i>	<i>Exc.</i> +1,000 →	\therefore <i>Volt.</i>	<i>Exc.</i> -1,000 ←		<i>Exc.</i> +10,000 →	\therefore <i>Volt.</i>	<i>Exc.</i> -10,000 ←	\therefore <i>Volt.</i>	$1/100.$	<i>R. x 2,000</i>
-0.0010	16	250	+ 5	+0.0031	+7	+0.0044	+ 8	+0.0030	+1	+0.0006	20	200
-0.0030	17	235	+15	+0.0088	+9	+0.0053	+22	+0.0130	0	0	20	200
-0.0030	20	200	+ 7	+0.0035	+2	+0.0010	+ 8	+0.0040	0	0	24	167
-0.0030	16	250	+ 7	+0.0044	+6	+0.0037	+12	+0.0075	+2	+0.0012	20	200
-0.0030	20	200	+ 7	+0.0035	+6	+0.0030	+28	+0.0140	+5	+0.0035	18	222
-0.0130	18	222	+24	+0.0133	+6	+0.0033	+20	+0.0111	+3	+0.0016	19	210
-0.0070	12	333	+12	+0.0100	+9	+0.0075	+12	+0.0100	0	0	15	267
+0.0070	29	138	+ 1	+0.0003	0	0	0	0	-3	-0.0010	29	138
-0.0040	20	200	+ 2	+0.0010	0	0	+11	+0.0035	+1	+0.0005	20	200
-0.0040	20	200	+10	+0.0030	+7	+0.0035	+18	+0.0090	+6	+0.0030	16	250
			<i>Av.</i>	+0.0032	<i>Av.</i>	+0.0032	<i>Av.</i>	+0.0080	<i>Av.</i>	\pm 0.0010		

In Table II the average results of five sets of experiments carried out at different times are tabulated :

TABLE II.

No.	No. of embryos tested.	Average electrical response in volts, strength and direction of shock being given at head of columns.				Date.	Direction of response.
		+1,000 units.	-1,000 units.	+10,000 units.	-10,000 units.		
1.	10	0.0023	0.0019	0.0074	0.0018	April 9	all +
2.	10	0.0051	0.0032	0.0033	0.0005	" 12	a few —
3.	10	0.0020	0.0017	0.0039	0.0002	" 14	all +
4.	10	0.0021	0.0016	0.0059	0.0013	" 19	a few —
5.	10	0.0020	0.0013	0.0033	0.0004	" 22	all +

Table II.—Average responses of embryos of *Hordeum vulgare* dissected from the grain after germinating for 30 hours on filter-paper soaked in distilled water at a temperature of 22° C. to 25° C. Average germination value = 85 %.

TABLE III.

Electrical response in volts. Strength and direction of shock at head of columns.

	+1,000 units.	-1,000 units.	+10,000 units.	-10,000 units.
	+0.0009	+0.0004	+0.0036	0
	+0.0021	+0.0025	+0.0035	0
	+0.0035	+0.0040	+0.0030	0
	+0.0040	+0.0028	+0.0050	+0.0011
	+0.0000	0	+0.0008	+0.0008
	+0.0028	+0.0017	+0.0066	0
	+0.0007	+0.0011	+0.0030	0
	0	0	+0.0040	0
	+0.0070	+0.0045	+0.0085	+0.0005
	0	0	+0.0017	0
Average of ten experiments:—	+0.0021	+0.0017	+0.0039	+0.0002

Table III.—Electrical responses of 10 embryos of *Hordeum vulgare* after treatment for 30 hours with distilled water. Temperature = 22° to 25° C. Germination value = 85 %.

TABLE IV.

Response in volts. Strength and direction of shock at head of columns.

	+1,000 units.	-1,000 units.	+10,000 units.	-10,000 units.
1.	+0.0020	+0.0020	+0.0033	+0.0013
2.	0	0	0	0
3.	+0.0030	+0.0020	+0.0060	0
4.	+0.0027	+0.0008	+0.0080	0
5.	+0.0150	+0.0125	+0.0025	0
6.	-0.0060	-0.0030	-0.0020	-0.0010
7.	+0.0083	+0.0050	+0.0025	-0.0012
8.	+0.0100	+0.0050	+0.0050	-0.0020
9.	0	0	0	0
10.	+0.0044	+0.0020	+0.0037	0
Average of ten experiments:—	±0.0051	±0.0032	±0.0033	±0.0005

Table IV.—Electrical responses of embryos of *Hordeum vulgare* after treatment for 30 hours with distilled water. Temperature = 22° to 25° C. Germination value = 85 %.

Tables III and IV are given as examples of the detailed experiments. It is noticed that the responses in Table III are all in the same positive direction. The responses in Table IV are also generally in this direction, but occasionally the direction changes to negative.

In Table V is shown the average results of three sets of experiments on another sample which gave 'a slightly higher' germination value.

TABLE V.

No.	No. of embryos tested.	No. of hours soaking.	Average electrical response. Strength and direction of shock given at head of columns.				Date.	Direction.
			+1,000 units.	-1,000 units.	+10,000 units.	-10,000 units.		
1.	10	30	0.0041	0.0029	0.0062	0.0007	April 26	+
2.	8	30	0.0025	0.0023	0.0059	0.0009	April 29	some -
3.	10	27	0.0052	0.0032	0.0079	0.0010	May 3	some -

Table V.—Average electrical responses of embryos of *Hordeum vulgare* after germinating on filter-paper soaked in distilled water at a temperature of 22° C. to 25° C. Germination value = 90 %.

These responses are noticeably higher on the whole than those in Table II, and the negative response only occurred in two cases in the total number of experiments.

(ii) Two sets of the same sample of grains were germinated under different conditions at the same time, and the electrical response obtained. In Table VI an example of the result is given. It was found that the use of culture solution instead of distilled water increased the percentage of grains showing signs of germinating energy in a certain time. There was a distinct relationship between this and the average electrical response.

TABLE VI.

	% germinating at end of 40 hours.	Average electrical response. Strength and direction of shock at head of columns.			
		+1,000	-1,000	+10,000	-10,000
1. Culture solution	76	0.0016	0.0013	0.0046	0.0023
2. Distilled water	40	0.0011	0.0013	0.0024	0.0014

Table VI.—Average electrical responses of embryos of *Hordeum vulgare* germinated for hours on moist filter-paper. Temperature = 20° C.

TABLE VII.

	Electrical response in volts.		Strength and direction of shock at head of columns.	
	+1,000 units.	-1,000 units.	+10,000 units.	-10,000 units.
1.	+0.0055	+0.0050	+0.0125	0
2.	+0.0041	+0.0012	+0.0024	+0.0012
3.	0	-0.0010	+0.0055	+0.0020
4.	+0.0014	0	+0.0023	-0.0014
5.	+0.0070	+0.0060	+0.0090	0
6.	0	0	-0.0012	-0.0017
7.	+0.0080	+0.0053	+0.0112	0
8.	+0.0013	+0.0035	+0.0026	-0.0013
9.	+0.0070	+0.0060	+0.0035	+0.0012
10.	0	0	+0.0008	0
Average of 10 experiments —	+0.0034	±0.0028	±0.0051	±0.0008

Table VII.—Electrical responses of embryos of *Hordeum vulgare* treated with distilled water for 28 hours. Temperature = 23° C. Germination value = 85 %.

TABLE VIII.

	Electrical response in volts.	Strength and direction of shock at head of columns.		
	+1,000 units.	-1,000 units.	+10,000 units.	-10,000 units.
1.	[-0.0025] +0.0019	+0.0087	+0.0062	-0.0025
2.	o	o	o	o
3.	o	o	o	o
4.	o	o	o	o
5.	o	o	o	o
6.	+0.0010	+0.0005	-0.0014	-0.0010
7.	o	o	o	o
8.	o	o	-0.0013	o
9.	o	o	o	o
10.	+0.0054	+0.0041	+0.0091	+0.0004
Average of 10 experiments:—	±0.0008	+0.0013	±0.0018	±0.0003

Table VIII.—Electrical responses of *Hordeum vulgare* treated with distilled water for 28 hours. Temperature = 23° C. Germination value = 50 %.

(iii) Using different samples giving different germination values—50 per cent. and 85 per cent. respectively. The results are most striking.

Tables VII and VIII are examples of ten experiments on grains which were treated with distilled water on filter-paper for twenty-eight hours under the same conditions of temperature, moisture, &c., the electrical response of both samples being recorded the same afternoon to minimize any difference in the magnitude of the response due to slight differences of temperature, atmospheric conditions, &c., at the time of experiment. It is to be observed that the much lower average electrical response in the case of grains of the lower germination value is due to the larger number of embryos giving no response to electrical stimulus. Thus the 50 per cent. germination grain gives no response in about half the experiments.

In Tables IX and X the record of the electrical responses in the case of the same samples of grain as above (85 per cent. and 50 per cent.) is tabulated. The galvanometric experiments were carried out when the grains had been soaked for twenty-five hours in distilled water at a temperature of about 25° C. Here this point is brought out—that there is probably a time of *maximum* electrical response for these embryos just about the period when growth becomes obvious. This time would vary with the sample, and it is to be expected that the better the sample the sooner this response would be given. This is obviously a point of the greatest practical importance, and having ascertained that there *is* a parallelism between germinative capacity and electrical response, further experiments along these lines will evidently attempt to answer the question of the practical expert: 'What is the *shortest* time at which the electrical response will indicate the comparative vitality of a sample of seeds?'

Table XI gives the average electrical responses of the four sets of experiments described above.

TABLE IX.

Electrical response in volts. Strength and direction of shock at head of columns.

	+ 1,000 units.	- 1,000 units.	+ 10,000 units.	- 10,000 units.
1.	+ 0.0065	+ 0.0040	+ 0.0150	+ 0.0030
2.	+ 0.0058	+ 0.0058	+ 0.0092	+ 0.0046
3.	+ 0.0045	+ 0.0043	+ 0.0220	- 0.0110
4.	+ 0.0100	+ 0.0100	+ 0.0100	0
5.	+ 0.0177	+ 0.0085	+ 0.0085	- 0.0030
6.	+ 0.0008	0	+ 0.0023	+ 0.0008
7.	+ 0.0263	+ 0.0173	+ 0.0127	- 0.0027
8.	+ 0.0025	+ 0.0090	+ 0.0115	0
9.	0	0	- 0.0020	0
10.	+ 0.0100	+ 0.0057	+ 0.0121	- 0.0021
Average of 10 experiments :—	+ 0.0124	+ 0.0103	± 0.0105	± 0.0027

Table IX.—Electrical responses of embryos of *Hordeum vulgare* treated with distilled water for 25 hours. Temperature = 23° C. Germination value = 85 %.

TABLE X.

Electrical response in volts. Strength and direction of shock at head of columns.

	+ 1,000 units.	- 1,000 units.	+ 10,000 units.	- 10,000 units.
1.	0	0	0	0
2.	+ 0.0050	+ 0.0040	+ 0.0150	+ 0.0025
3.	0	+ 0.0004	0	0
4.	+ 0.0020	0	+ 0.0025	+ 0.0005
5.	0	0	0	0
6.	0	0	+ 0.0004	+ 0.0004
7.	+ 0.0100	+ 0.0220	+ 0.0240	+ 0.0010
8.	0	0	0	0
9.	[- 0.0012]	0	0	0
10.	+ 0.0024	+ 0.0016	+ 0.0040	+ 0.0012
Average of 10 experiments :—	+ 0.0013	+ 0.0040	+ 0.0073	0
	+ 0.0021	+ 0.0032	± 0.0053	± 0.0005

Table X.—Electrical responses of embryos of *Hordeum vulgare* treated with distilled water for 25 hours. Temperature = 23° C. Germination value = 50 %.

TABLE XI.

Electrical response in volts. Strength and direction of shock at head of columns.

Germination value.	+ 1,000 units.	- 1,000 units.	+ 10,000 units.	+ 10,000 units.	No. of hours germinated.
85%	0.0034	0.0027	0.0051	0.0008	28
85%	0.0124	0.0103	0.0105	0.0027	25
50%	0.0008	0.0013	0.0018	0.0003	28
50%	0.0020	0.0032	0.0053	0.0005	25

Table XI.—Average electrical responses of embryos of *Hordeum vulgare* treated with distilled water. Temperature = 23° C.

SUMMARY OF RESULTS AND CONCLUSIONS.

1. The same samples of grain germinated under approximately the same conditions give results in which the germination value and the electrical response are quite consistent.

2. The electrical response of grains germinated under different conditions, such as food supply, temperature, &c., varies in the same way as the germination value.

3. Samples of grain giving good and bad germination values give average electrical responses which vary strikingly in the same way.

4. There is a certain amount of evidence that there is a time of maximum electrical response, probably corresponding with the time when growth becomes established. Experiments are being carried out to determine this point with more precision.

5. In the samples giving a low germination value there are always a certain number of embryos which give no response to the electrical stimulus, indicating they are incapable of germination. A high proportion of such zero results under conditions which would normally produce a response would indicate a sample of low germinative value.

6. The electrical response can be ascertained in a much shorter time than the total germinative value.

7. There is an indication that the electrical response would discriminate not only between a 'live' seed and a 'dead' seed, but between a 'live' seed of high vitality and one of low vitality.

I take this opportunity of expressing thanks to the Board of Agriculture, with whose assistance this work has been carried out.



NOTES.

ANOMALIES IN THE OVARY OF *SENECIO VULGARIS*, L.—

In all the species of Compositae examined hitherto, with the exception of *Taraxacum officinale*¹ and *Zinnia* spp.,² only one ovule has been reported in each ovary. Schwere¹ figures two ovules with embryo sacs in *T. officinale*, but in this case they were apparently basal. In the present investigation of *Senecio vulgaris* several abnormalities have been observed which are of phylogenetic importance. The occurrence of biovulate ovaries was noted in several instances, two of which are figured (Figs. 1 and 2). In the former case the archesporial cell is quite distinct, and a wall two cells thick extends across the ovary. The occurrence of a biovulate, bilocular ovary is interesting confirmation of the derivation of the unilocular ovary of the Compositae from the former type. Both ovules in this case were on

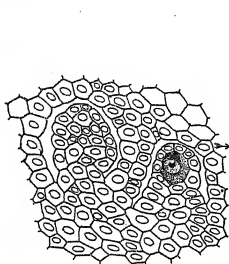


FIG. 1.

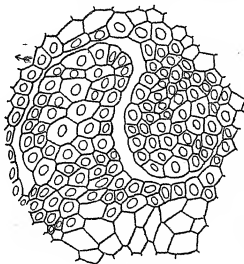


FIG. 2.

one side of the ovary, but in the latter case (Fig. 2), where there is no wall, the ovules are placed on either side of the ovary. The four ancestral placentae are thus indicated. In both cases the ovules are lateral. Numerous cases of single lateral ovules were observed in the younger stages (Fig. 3), and this is an abnormality of some importance. Particular care was exercised in determining that these ovules were truly lateral; the presence of conducting cells and the arrangement of the adjacent parenchyma leave no doubt as to the actual attachment of the ovule to the side wall of the ovary. The arrow in the figures indicates the position of the axis. Fig. 3 is a longitudinal section, tangential to the axis.

¹ Schwere, S.: Zur Entwicklungsgesch. der Frucht von *Taraxacum officinale*. Flora, vol. lxxxii, 1896.

² Don, D.: On the Origin of the Ligulate Rays in *Zinnia*. Trans. Linn. Soc., vol. xvi, 1829. [Annals of Botany, Vol. XXX. No. CXVII. January, 1916.]

Warming¹ removes the Calyceraceae from the vicinity of the Compositae, and places them near the Dipsaceae on account of the ovule, which he distinguishes as 'apotrope', i.e. placed on the anterior wall of the ovary with the raphe anterior. He argues that if the basal ovule of the Compositae, which he designates 'epitrope', were displaced so that it was pendant, the raphe would become posterior, instead of anterior as it is in the pendant ovules of the Dipsaceae and Calyceraceae. This assumes that the displacement is in the median plane of the flower, but the present observations of the orientation of lateral, i.e. displaced, ovules show that displacement takes place in the plane at right angles to the median plane. Therefore, if the basal ovule of the Compositae were displaced so that it became pendant, the raphe would remain anterior and the ovule would have the same position as in the Calyceraceae and Dipsaceae. This displacement in the lateral plane is just what might be expected, considering the obvious derivation of the unilocular ovary of the Compositae from a bicarpellary ovary in which the carpels were on the antero-posterior plane. Therefore the Calyceraceae, in accordance with the usually accepted opinion, may be allowed to remain near the Compositae.

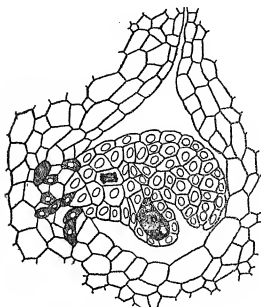


FIG. 3.

Van Tieghem² also removes the Calyceraceae from the vicinity of the Compositae, and places them near the Rubiaceae on account of the ovule, but the value of the ovule in the classification of this portion of the Sympetalae has been somewhat over-emphasized considering the comparative frequency of these lateral ovules, which form a transition to the Calyceraceae and Dipsaceae.

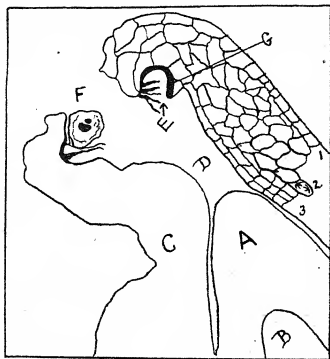
JAMES SMALL.

ARMSTRONG COLLEGE,
October, 1915.

¹ Warming, E.: Observations sur la valeur systématique de l'ovule. *Mindeskript f. Japet. Steenst.*, 1913.

² Van Tieghem, P.: L'œuf des plantes. *Ann. sci. nat., Bot., sér. 8*, t. xiv, 1901.

NOTE ON THE STRUCTURE OF THE OVULE OF *LARIX LEPTOLEPIS*.—The writer began the study of the structure and life-history of the various Larches in March 1915. Such a study seemed profitable, as, except for occasional observations, the group has been practically neglected. Even such references as do exist are mostly detailed cytology, like Allen's admirable account of the formation of the spindle in the reduction division of the pollen mother-cells of *L. europaea* (Ann. Bot., vol. xvii, 1902). But the broad outlines of the life-history are still far from ascertained. Unfortunately, the work has not made sufficient progress this season to warrant a complete account, and this for many reasons. The absence of information on the group meant that the dates of the appearance of the various stages were unknown, and it was thought advisable to determine these dates as a preliminary to further work. The abnormal sterility of the specimens at the writer's disposal was also a hindrance. It was no uncommon thing in July, when removing the hardened



Longitudinal section of the upper part of the ovule of *Larix leptolepis*. A, nucellus; B, embryo-sac; C, integument; 1, 2, 3, its layers in process of differentiation; D, micropylar constriction; E, fold with stigmatic hairs; F, a pollen-grain entangled; G, thickened plate from which hair-like processes arise.

integument, to cut into 200 ovules before meeting a good one. Finally, difficulties in procuring fixing chemicals, &c., owing to the war, also retarded progress. But there were one or two points which appeared which it is considered well to put at once into this preliminary note.

I. The structure of the ovule was interesting and peculiar. Most worthy of record, however, is the fact that its structure was almost identical with that described by Lawson for the ovule of *Pseudotsuga Douglasii* (Ann. Bot., vol. xxiii, 1909). To describe its appearance and to emphasize the fact of its similarity to that of *Pseudotsuga* nothing can be better than to follow Lawson's description of his ovule on the accompanying figure, which is a longitudinal section of the upper part of the

ovule of *Larix leptolepis*. Lawson says: 'The pollen-receiving device in *Pseudotsuga* is quite peculiar and unlike anything yet described for Gymnosperms. For some little time after pollination the nucellus presents the form of a small protuberance with a perfectly rounded apex (A). The integument (c) extends for a considerable distance beyond the nucellus. At a point immediately above the apex of the nucellus the integument bends inwards in such a fashion as to partly close or narrow the micropylar canal, and then sharply bends out again. This results in the formation of a distinct stricture midway between the apex of the nucellus and the mouth of the micropyle (n). As a result of this peculiar curvature of the integument, the micropylar canal is not a straight passage of uniform width, but consists of two chambers, one immediately above the apex of the nucellus and the other near the mouth of the micropyle. In addition to this narrowing in the middle region of the micropyle, the integument is still further modified. The extremity of the integument which forms the mouth of the micropyle is folded inward (e). On the inner surface of this enfolding extremity numerous fine hair-like processes are present. A close examination of these processes makes it clear that they were not cellular in structure, but were merely outgrowths from the external walls of the epidermal cells. They serve very effectively, however, as a stigmatic surface.' The hairs in *Larix leptolepis* are firmer than those figured by Lawson, and arise from a basal plate (e). He goes on to say that pollen-grains were never found on the nucellus: 'They were invariably found in the upper chamber of the micropyle and frequently entangled in the hair-like processes of the mouth.' A similar condition is found in *leptolepis*, the figure showing a pollen-grain so entangled (f).

The quotation shows how like the two ovules are. The explanation may be, and most probably is, biological, but may also have some phylogenetic significance. The megaspore membrane in *leptolepis* does not cover the upper end of the endosperm, a condition similar to *Pseudotsuga*. There are very small archegonial chambers in both. Lawson states the frequent presence of only one tier of neck-cells in *Pseudotsuga*. In *L. leptolepis* the prevailing condition seems to be one tier of five cells. There are typically five archegonia in *L. leptolepis*, four to six in *Pseudotsuga*. All these points are, of course, of minor importance, but with the peculiar sameness of the ovules there is an obvious temptation to magnify them. The detailed investigation may, however, settle the point.

II. The archegonial jackets usually touch, so that two archegonia are only separated by two cell-layers. Frequently these coalesce to one, and even the archegonia may be separated only by the shrivelled remains of degenerated jacket-cells.

III. Double pollen-grains are very plentiful in *L. leptolepis*, as already described by Coker (Bot. Gaz., vol. xxxviii) for *L. europaea*, by Hutchinson (Bot. Gaz., April, 1915) for *Picea*, and others. Their origin will be investigated next spring, as, even though material was first collected on March 15, practically all the pollen was shed on March 16, in spite of the fact that the spring was a phenomenally late one. *L. europaea* presents similar features.

IV. There is one last point—a vegetative abnormality. As is well known, the male buds of the Larch appear terminally on dwarf shoots from the second year on. While collecting such buds—already well developed in August—a case was noticed of a dwarf shoot carrying such a bud. The shoot was six years old, judging by the

leaf-scar rings on it. Growing out from the dwarf shoot from the axil of the third-year ring of leaves there was a very small secondary dwarf shoot with six or seven small leaves on it. Such an appearance is quite understandable, because, if a dwarf shoot under suitable conditions can become a long shoot upon which dwarf shoots are subsequently formed, there is no reason *a priori* why secondary dwarf shoots should not appear on an ordinary dwarf shoot. But the branching of dwarf shoots in the Abietineae has not often been described.

Special thanks are due to Sir Frederick Moore, of the Glasnevin Gardens, Dublin, who has placed several Larch trees entirely at the writer's disposal.

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DUBLIN.



Yrs ever
D. T. Swaine-Vaughan

Annals of Botany, vol. xxx.

David Thomas Gwynne-Vaughan.

With Portrait.

DAVID THOMAS GWYNNE-VAUGHAN¹ was born on March 12, 1871, at Royston House, Llandovery, his mother's home. He was the eldest child of his parents, Henry Thomas Gwynne-Vaughan, of Cyngordy, and later of Erwood Hall, Breconshire, and Elizabeth, second daughter of David Thomas, of Royston House, Llandovery, who died in 1874. His father, who had two daughters and a son by a second wife, died in 1890.

The Vaughans are an ancient Welsh family, descended from Sir Roger Vaughan, who was killed at Agincourt, and was one of the 'three valiant Welshmen . . . who had rescued the King, and were knighted by him as they lay bleeding to death'.

Going back to more mythical days the Vaughans trace their descent to Cradoc the Strong Arm, one of the Knights of the Round Table, and further back still. In remote days the Gwynnes of Cyngordy and the Vaughans are said to have had a common ancestor in Aulach, great-grandfather of Cradoc, who again was the descendant of Gwarldeg, King of Garthmadryn (now Brecon) (A. D. 230?).²

That extraordinary person Thomas Vaughan, the Rosicrucian and alchemist (1621-65), belonged to another branch of the family.

David Gwynne-Vaughan attended a preparatory school at Kington, Herefordshire, and later, the Monmouth Grammar School.

In the October term of 1890 he entered Christ's College, Cambridge, with an exhibition from his school, and in the following year obtained a scholarship in Science from the College. He was thus a member of Charles Darwin's college, an association which, as it seems to me, had a special appropriateness. He took a First Class in Part I of the Natural Science Tripos in 1893.

Gwynne-Vaughan did not go on to Part II of the Tripos, and thus had no opportunity at Cambridge of showing his real powers. During the year after he left Cambridge, he was engaged in teaching, as Science Master at a school.

¹ I am indebted to Mrs. H. C. I. Gwynne-Vaughan, D.Sc., F.L.S., for particulars of her husband's life and family, and for much kind help in other ways.

² I understand that much more information about these old families is to be found in Jones's History of Brecknockshire and Nicholas's Annals and Antiquities of the Counties and County Families of Wales.

I first became acquainted with Gwynne-Vaughan in the autumn of 1894. It was at the invitation of the Director, Sir W. T. Thiselton-Dyer, that he came to work at the Jodrell Laboratory, of which I was then Honorary Keeper. After a couple of preliminary visits he started work on Oct. 12, beginning, by way of practice, with an examination of the roots of *Pandanus*. We used to go round the houses and pits together, and he soon became familiar with the treasures of Kew. He made a good many observations on Cycads, and took part in the hunt for centrosomes, on which in those days we were all bent, led by the flattering tale which had been told so well.

Gwynne-Vaughan's skill as a microscopist soon became evident, and from the outset he was very keen. For the first three months or so his work was rather miscellaneous—he was getting into training. In January, 1895, he began to settle down to definite research. His first subject was the anatomy and morphology of Nymphaeaceae, on which he made some curious and interesting observations, afterwards embodied in his Notes of 1896 and paper of 1897, referred to further on.

Kew provided him with some specially interesting material for his work; in particular, a whole series of seedlings of *Victoria regia*, at successive stages, was placed at his disposal. In suggesting the group for investigation, I had specially in view the peculiarities of its anatomy, to which Gwynne-Vaughan was soon able to add some new and unexpected features; at the same time his attention was further directed to points in the external morphology.

A little later, in May of the same year, he took up another anatomical subject, the structure of polystelic *Primulas*; as the result of his observations he was able to make a considerable advance on the views of Van Tieghem.

About this time there was some question of his going on an expedition to South Brazil, but it was not till a couple of years later that his hopes of tropical travel were realized.

In September, 1895, Gwynne-Vaughan attended the meeting of the British Association at Ipswich, the first at which a separate Section for Botany was constituted.

On October 8, 1895, W. H. Lang (now Professor of Cryptogamic Botany in the University of Manchester) first came to the Jodrell Laboratory. This was the beginning of the close and life-long friendship between him and Gwynne-Vaughan.

In November of that year Gwynne-Vaughan showed me the draft of his Nymphaeaceae paper, the first written product of his work; it was considerably extended before publication, and some of the most interesting results were recorded meantime in two Notes in the 'Annals of Botany' (1896).

Lang was working at apogamy in Ferns, and on August 20, 1896, made his astonishing discovery of the occurrence of sporangia on the prothallus. Gwynne-Vaughan was present, and when they told me what Lang had found, I did not believe a word of it, but thought they were 'chaffing'. However, it was quite serious, and within a few days another case of the same kind came to light in a different Fern. I mention this here, because Gwynne-Vaughan and Lang were working in the same room, and the former was just as keen on Lang's results as the discoverer himself.

In September, 1896, Gwynne-Vaughan was present at the Liverpool meeting of the British Association, and this visit had an important influence on his after career. He read a paper on 'The Arrangement of the Vascular Bundles in certain Nymphaeaceae'. Professor Bower was impressed by this 'peculiarly lucid preliminary statement', and at once offered Gwynne-Vaughan the Junior Assistantship in his laboratory at Glasgow. If Kew may claim to have first started Gwynne-Vaughan on original work, it was at Glasgow that he developed his most characteristic line of research.

During the ensuing autumn Gwynne-Vaughan again worked at Kew, completing his paper on Nymphaeaceae and his investigation of the anatomy of *Primula*. The former was passed for publication by the Council of the Linnean Society on Dec. 17. Next day I said good-bye to him at Kew, and with the New Year (1897) his Glasgow career began.

It was not long, however, before his energies were diverted for a time into other channels. He accepted a commission to undertake a journey in South America, up the rivers Amazon and Purus, to report on the Rubber production of the district for a commercial syndicate, starting on Oct. 9, 1897.

He travelled 2,500 miles up the great river and its tributaries, and reached the Bolivian frontier.

I am permitted to make some extracts from letters written on this and on the subsequent Malayan journey, to his half-sister in England. The first is from a letter, written between Obidos and Serpa, and post-marked 4/12/97:

'But the journey up the Amazon is the most fascinating thing I have yet experienced. In parts we steam along a biscuit toss from the margin of the stream, which is bordered on either side by a solid wall, 60 ft. high, of virgin, impenetrable Forest of the most luxuriant tropical vegetation. Palms of various kinds stand out in delicate relief against the darker mass of giant trees of India-rubber, *Mimosae*, *Bertholletia*, &c., and the very water's margin is crowded with gigantic plants of the *Arum* family, reaching even to 20 ft. high. It is vain to attempt to describe the positively wanton prodigality of nature in these regions. Most of this Forest

is 2-6 ft. sunk in water for the extent of many miles on either side of the free stream.'

'Every common weed in the streets is completely new to me, and the size and beauty of the flowers in the waste spaces are very impressive—every one is new to me, and I am feeling very confused with it all, when I think that the wealth of the Virgin Forest has yet to be encountered.'

The next extract is from a letter written just after his return to Kew :

'For a description of the journey along thousands of miles of river, flanked by ancient Forest over 100 ft. high, the strange beasts in the water and on the land, the occasional settlements we met with, and their inhabitants, the Indians almost white and quite pleasant fellows that we came across, the miles that I have walked in the half-flooded, damp, and heated "Gapo" or low-lying Forest, with the thermometer at 90° in the shade, alone with a single Indian guide and a flask of the local "40-rod exterminator"—of all these anon. Of snakes and sun—but as Rudyard would remark, "That's another story". These I must keep until the fortunate hour in which we next meet.

'Myself and my companion were travelling against time, so I'm sorry to say my botanical results were only meagre, and I am haunted by an appropriate quotation :

"Ah, fool was I and blind ;

The worst I stored with *utter toil*,

The best I left behind."

I'm going to fire this off at a good many ; an apt quotation is the best sort of excuse.'

On Sept. 16, 1898, he was back in England, and returned to Kew to work for a time at the laboratory again. During this visit he was doing the anatomy of *Loxsoma*, and afterwards of other Ferns, for his solenostely papers. We had many discussions on questions of stelar morphology and the comparative anatomy of Ferns. He stayed at Kew over Christmas, leaving on Jan. 3, 1899.

The following month he started on his second tropical journey, that to the Malay Peninsula. It is characteristic of him that the interval between these two adventurous expeditions was filled up with regular laboratory work.

From *The Times*, Spring, 1899 :

'*Cambridge Expeditions in the Far East.*

'An expedition, under the leadership of Mr. W. W. Skeat, left Cambridge a few days ago for Bangkok. The members of the party include Mr. Gwynne-Vaughan, of Christ's College, and Messrs. Evans & Annandale, of Oxford, and it will be reinforced at Singapore by Mr. Bedford, of King's College.

The object of the expedition is to make a scientific survey of the little-known country lying south of Siam and north of the protected States of the Malay Peninsula.

As his experiences on the Malayan trip were of considerable interest, rather full extracts from his letters, above referred to, are given.

Approaching Singapore, March 8, 1899.

'I dread Singapore; we have an appalling number of engagements to make there, in the least possible time. If nothing else, you have a peaceable time at sea, a time that I love, though Malayan has done its best to spoil this trip as Portuguese did my former one; still I live in hopes of returning. If I do I am afraid this will upset the last chance of settling down and quiet work in England. I greatly fear the East, for was I not born a tramp-Royal?'

As we shall see, this anticipation was very far from being fulfilled.

Bangkok, Siam, March, 1899.

'I have dined at a Siamese Prince's, Prince Naret, in dress clothes and a stick-up collar, eaten a splendid European dinner, conversed with his wife, who is a delightful little person, but she chews betel to such a degree that an average European would have a fit, and talked over the odds on the Varsity boat-race with his son, Prince Charoon, an old Cambridge man and a very nice fellow too, while looking on at a Siamese dramatic performance commanded at the Prince's house for our benefit, and that of the officers of a British warship at present in port.

'Last night we dined on board the same, after having spent a long day at Ayuthia, the former capital of Siam, a wonderful place, a city which formerly contained about a million inhabitants, but now only a few majestic ruins scattered among a jungle only penetrable with difficulty. Huge palaces and temples, an image of Buddha 40-50 ft. high, and sitting at that, alone, huge, stately, and impassive in its vast unroofed and crumbling temple, while the irresistible jungle is steadily covering, destroying, and breaking down all things around it. I now know, as few do, what is meant by the "The Letting in of the Jungle". I stood in the hand of this gigantic idol and declaimed "The Karela, the bitter Karela", &c.

'The Malay is grievously misjudged in England. I distinctly like the race. They are a folk who are proud and sensitive, the only race in the East who look you straight in the face when they speak to you. In their manners they are naturally gentlemen. They admit a possible superiority in Englishmen, but hold all the Eastern races in strong contempt. It is true that they have some excusable little eccentricities, such as running amok and using their kris too freely, but then no race is altogether perfect.'

Singora, April, 1899.

'When we landed at Singora we arranged for some boats and a dozen Siamese boatmen to take us and our band of brigands up the Ta Lei Sap, a large inland sea here which only two Europeans have ever seen before. This we did, thanks to our credentials from the Siamese Government, pretty comfortably, and having reached a village half-way up we started our collections. Then the party divided. I and another one, Evans, started in the boat, and the other two went inland to investigate a tribe of very primitive savages with peculiarly repulsive and interesting methods of burying. I and Evans went in search of another lake to the north of Ta Lei Sap, called Ta Lei Naw, found and entered it, being the first Europeans to see that fine sheet of water. That night I attained the Ultima Thule of a traveller's desire, that is, I reached a spot where I was the very first white man to tread the soil. I should like to draw you a picture of our evening meal, after thirteen and a half hours' fast too, as we sat and wolfed, with a ring of about seventy natives seated around at a respectful distance, and observing us with interest and sundry guttural remarks. My gun caused considerable comment next day (I do the shooting of the Expedition, and do it extremely well). N.B. I have had a lot of luck in shooting lately, and must "blow" a bit. I had some difficulty in doing much there, because the *whole* village insisted on accompanying me, and made enough noise to frighten all the game into the next continent.'

Biserat in Falor (undated).

'Here I have made the acquaintance of a most villainous old brigand in a village near a mountain just here; he has instituted himself as my companion and mentor in shooting excursions and ascents of the hill, and he has just shown me, as a great treat, the entrance of a cave which runs direct into the hill. We have been yearning for caves for some time, and I am very pleased, because the very next day I got torches and, accompanied by my hired assassin, we have explored it. He, the betel-chewing old assassin in question, is the only man here who dares enter, because there are a large number of exceptionally ferocious and horrible "Hantus" (Demons, or Devils, mixed up a good deal with ghosts). A strong wind comes out from the mouth of the cave, which is their breathing. The brigand did his utmost to dissuade me for my own good, advancing numerous weighty reasons, such as that the cave ended in a pit which dropped through to the infernal regions, and that it was not the right time of year. Somewhat incongruous, isn't it? He only screwed himself up to accompany me by the offer of bullion, and by assurances that I had paid special attention to cave-demons, and was capable of coping with any likely to appear; indeed, I openly confessed to intimate acquaintance with several cave-devils of the higher class. Further, he besought me to bring a little

stick (very ordinary stick) that I have had given me, and which is universally known to be peculiarly efficacious for almost all kinds of Hantus. This reads funny, but the old boy genuinely believes it, or fancies he does. We said charms before we entered, both of us. Mine (the genders of Latin nouns ending in -is) pleased him vastly, and he would very much like to learn it, especially when I told him that it could be used in other cases too, as well as cave Hantus, especially when prefaced with

"Feminine -do -io -go -is, -as -ans and -x, will show

-Es, if no increase be needed, -s by consonant preceded".

The cave turned out to be really fine, with magnificent Cathedral-like domes, far into the mountain. Since then I have found a simply majestic Cathedral cave, with a small opening at the top and others imitating clerestory windows, the floor strewn with huge stalagmites imitating white marble tombs. I have never seen anything like it. I also found a deep pot-hole, which I descended by a rope. I got about 150 ft. down, when it stopped slanting and dropped sheer. I laid myself out on a tooth of rock overhanging the black mouth of the pit and dropped [a stone?]. I counted ten before it struck bottom, and simultaneously became nervous about the stability of the jutting rock, thought of Rider Haggard, and precipitately retired. I was very, very careful in climbing back up, and I told the brigand that the Hantu at the bottom of this cave was a very big and dangerous one indeed. He instantly agreed and said that he had seen him once, something like a goat and something like a man, and very big. I said he was quite right, and that we'd better leave him alone till I'd worked up a suitable charm to floor him with. Apart from this personal interest, the caves have a scientific one, for we have discovered a "cave fauna" in them, about twenty species, including a snake which we caught in a butterfly net, yet he was 6 good feet long, and probably very poisonous. All these never saw the light, being sightless. They feel their way in continual darkness, existing in a strange little world of their own. Highly interesting, isn't it?

'I have been deer-hunting, pigeon-decoying, bull and cock fighting, and all sorts of weird things here. I hope, when Skeat arrives, to get a wizard I am acquainted with, to give an exhibition of devil-raising. I do not say these things flippantly. There are some *very strange* things out here among the jungles, cliffs, and caves.'

Kota Bharu, in Rahman, c. June 15, 1899.

'I like the Malays very much indeed; we have long talks at night, and they tell me all the ghost stories, and about "Hantus", Spirits, and Jinns, and all sorts of Devils. They have an enormous mythology which they really believe, with the weirdest and most incongruous conceptions imaginable.

'They admit, and really believe, that I am an advanced devil doctor (Pawang) myself. I barefacedly claim this honour. I have been away in a Malay hut, half a dozen waist-bare folk variously squatting on mats, in a closed space some 8 foot square, lighted by a couple of smoky dammars, all of us smoking or chewing betel, and having chanted and tambourined our nerves into a high state of tension, I have seen a brother Pawang drum himself into a positive epileptic fit, uttering short screams and mirthless laughs, throwing about his head, and twisting his limbs in a most unearthly manner, while we rhythmically drummed and incanted all the faster. The statements he made when in the fit were acted upon with the intention of amending the sick person whom we were professionally attending. It is known that devils had entered into the Pawang while in that state, and while he is coming to they speak through him. I must say he made a tolerably close approach to my preconception of demoniacal possession, but when the devils spoke in friendly and appreciative terms of myself, I was forced to confess it was not the real thing. They said that they had informed me of the best medicine. I stated that I *had* been informed, and that it was well. Then, since the person had dysentery, I administered chlorodyne, produced a rapid cure, and by the Pawang himself (who really seems half to believe it *all*) I am believed to control devils of extraordinary capabilities. I have been told that there are folk who would very much like to see my devils enter me. But I refuse because I am aware that I could not anything like approach the performances of the native professionals. I explained that my "Hantus" are exceedingly shy ("malu") of entering any one, and they only enter me under the most persuasive circumstances. Further to keep up their reputations, that if they, by mistake, entered any one else, they would almost certainly "bust" him.

'This may appear rather nonsense to you, but it is good sound, rational, and believable stuff out here, based on a knowledge of the popular opinions and surmises. It isn't Alice in Wonderland, though it is rather like.'

Kelantan River, July 26, 1899.

'Twice Hadji Sirath and I have had to work the raft on alone, half the time wading and half punting it along. The river is full of rapids; several times I have passed over rapids or waterfalls of 4 foot fall in almost as many yards, the passage being between rocks hardly apart enough to allow the raft to pass; twice indeed the bamboos of the raft touched on both sides, and once indeed even mounted the rock at one side. Between the wild swirl and rush of the waters, the swift descent of the raft twisting and turning with all its bamboos creaking and splitting, our cries of encouragement and warning, the waving of poles and the splashing of water, these rapids make the most exciting sort of time in their shooting. I would like to have a photograph of our descent immensely. If we made a really bad

shot, the rafts would come instantly to pieces and all my kit would probably be ruined. No other kind of craft would stand it. These rafts are only made of a few bamboos lashed together by rotan, and they bend like fishing rods before they break. Twice we have had to moor out our kits and descend in the raft alone, because it was too risky.

'Hadji and I sleep together at night on the raft, wherever night overtakes us, which it has up to now in the middle of the jungle. When it rains we put up my waterproof sheet, and go on getting wet as before. You will hardly believe it that, in spite of all this, I am in uproarious good health, and am enjoying it immensely, although I am frightfully savage at having the rest of the trip spoiled.'

'Now Tomoh is a gold country, worked by a Chinese colony, and one day on the march through it we changed our path from the bed of a large stream to a path (hardly better) through the jungle, when after a while we were petrified by meeting in the midst of the forest a little locomotive engine, and one or two trucks with English gold-mining machinery in them, all broken and decayed, the plates of the engine all burst and broken in all directions, but with its little rusty smoke-stack still erect. There it stood, as though suddenly slain in the midst of action, and now the forest had rapidly overgrown, crowded in and suffocated it, a stout liane twining round the smoke-stack painfully suggesting strangulation. The trail of the white man stood before us, the little engine looked pitiful and melancholy, and it felt as though we were deserting a disabled comrade in the face of a relentless and cruel foe, when we went on, leaving the work of our hands alone again and utterly desolate, to suffer the revenge of the violated jungle. The tale came out later on. Some time ago six "Orang putih"—whites—came here to work for gold. They stayed three years, they found none worth working, and then three of them went back, two died of fever and one by the fall of a rock. They must have been pretty good men because they left a good reputation among the Malays, who speak well of them.'

'This morning, after great difficulty in obtaining two men, we floated down on our raft very slowly, using paddles, the water now having become too deep to punt, until I got to Kluat a village. There I managed to buy a small prahu, a much swifter craft. Now there are no more rapids, the river being a fine one, about 150 yards wide, running through a hilly and densely afforested country, every now and then coming to a solitary house or Kampong. It is really excessively beautiful, and not in the least monotonous, as was the Amazon. I also managed to get three men to paddle, and actually got them to work nearly all night, chiefly because I could not sleep myself, since the boat leaked, and everything got soaking wet, to say nothing of the rain. We have made excellent progress, and I expect to get to Kelantan by night. One of the men I picked up is a certain Hadji Said,

a wicked old chap who has helped me a great deal, and undoubtedly stolen my pyjama coat. We have become great friends, although he prays fervently and by mention each night, that my head may burn violently in Jehanum. Islam is a frightfully uncompromising religion.'

The following extract from Gwynne-Vaughan's Journal gives an account of an adventure in the Malayan forest:

Near Tremangan.

'The jungle consisted of fairly high trees, and below a swamp, thick-set with *Pandanus*; we had to cut every yard of our way through. I had Akib and Uda with me, and we took turns at leading the way and using the parang. We were waist-deep in very malodorous swampy mud, swarming with leeches, trying to stand on *Pandanus* stumps and crawling along fallen and rotten tree-trunks, every now and then missing our footing and slipping into the horrid, repulsive mud over our middles. The *Pandanus* leaves cut our hands, lianes tripped us up, and the forest mosquitoes and leeches did their utmost to add to our discomfort, and a very melancholy spectacle we made when we got out into a clearing.

'One afternoon while here Akib and I got into a nasty adventure. While in a large padang, about its centre, we looked up and saw the leader of a herd of krebau, which had been grazing in the distance, had approached us uncomfortably near and looked distinctly uneasy. We tried to scare it off without result; at last it stamped its foot impatiently, Akib yelled out "diamon terkan" and lit out for the nearest jungle in fine style, and the krebau made for us; the whole herd, which had quietly crawled up close, also charged at his heels.

'I did a lot of quick thinking, during which I recollected I had only one shot cartridge in my gun, and then I took after Akib, being just in time to see him take a flying leap into the jungle. I put in a record 100 yards, getting home a little to the right of Akib, who, as I shot past, I observed was shinning up a tree as hard as he was able. I went on through the jungle, once nearly knocked down by my collecting tin getting stuck between two trees, and fortunately got through into another padang through which I cut at half-mile pace only. At the edge of the next jungle I stopped, and Akib shortly joined me, saying that the krebau had followed me and had not gone through far. I would have much liked to climb a tree myself, but I had the gun to look after.'

Gwynne-Vaughan was back in London on September 26, 1899, having been absent a little over seven months.

Caledonian Hotel, Strand, September 26, 1899.

'I am glad to get back of course . . . but really I feel strangely that I am an outsider looking on at the show, and hardly having any definite

part in it to play, my interests being still right away back with the honest, clean, and simple savagery of the Malay.'

His friend Lang, who was himself in the Malay Peninsula a year or two afterwards, writes to me as follows on the subject of Gwynne-Vaughan's travels:

'The expedition, as you know, had a pretty adventurous time in the unexplored Siamese States as well as in better-known Eastern States of the Peninsula. The experience made a deep impression on Gwynne-Vaughan's life and thought, but not on his work, which he continued on the straight line on his return. Apart from collecting for the expedition and loyally handing over all the results, he drank deeply of the life with the Malays, who were after his heart in many ways. The Malay attitude to many things was always with him afterwards, and since I was there, a little later, we kept up an interest in the subject and for a time hoped to return. Malay proverbs and phrases were always coming from him as the appropriate expression of his thought in circumstances little dreamt of in the East.

'The Amazon expedition was a much rougher and less scientific experience, but he was younger, and got great joy of adventure in pulling through tight places in which the whites were often more savage than the natives. The experience appealed to his joy in adventure, which was always a current parallel to that of his almost too conscientious discharge of the duties of his various posts.

'This side of him came out in his fishing expeditions to out-of-the-way spots in Ireland and the Hebrides, where he loved to meet with all sorts and conditions of men, and not to be suspected of having been "academic".'

There is nothing to add to these words, which seem to me to express perfectly the significance of Gwynne-Vaughan's journeys in his life.

After he returned from the Malay expedition he took up the thread of his work, where he had dropped it, and his most important publications date from the period which then opened. These will be considered afterwards; his career may be first shortly sketched. He remained at Glasgow till 1907—ten years in all. He assisted his Professor in preparing the second edition of the '*Practical Botany for Beginners*', which appeared in 1902 under their joint names. I believe this was the only work in book form in which he took part.

In 1904 he became lecturer in Botany at Queen Margaret's College, Glasgow, in addition to his other University duties.

In 1907 his long and happy association with the University of Glasgow came to an end, and he accepted the post of Head of the Department of Botany at the Birkbeck College, becoming a Recognized Teacher and Internal Examiner in the University of London. He did not, however, remain for very long in the ranks of London botanists, for two years later, in 1909, he became Professor of Botany in the Queen's University at

Belfast, a post which he held for five years. On his appointment he wrote to me :

‘It will be a great change at first from the “intensive” kind of work we do here in London. In spite of the much too hard work I have had to do here, I shall leave London with great regret. I am very fond of the South.’ He speaks also of the ‘keen and appreciative students’ he had at the Birkbeck.

Although somewhat isolated from his botanical colleagues, he was happy in his relations with his fellow professors at the University of Belfast. He accomplished much good work while there, especially continuing the joint research with Kidston on the Fossil Osmundaceae, begun at the end of the Glasgow period.

On December 7, 1911, he married Dr. H. C. I. Fraser, the distinguished cytologist, who had succeeded him as Head of the Department of Botany at the Birkbeck, a post which she continued to hold during their married life and still occupies.

In July, 1914, to the great joy of his friends in England, he accepted the chair of Botany in University College, Reading, which unhappily he was only destined to hold for about a year.

At Belfast the teaching work had been much concentrated in the Summer term, and was then very hard; at Reading it was more spread over the whole year, and would probably have proved less exacting when he had once got settled in the new position.

He had long been seriously troubled with neuralgia, though few of his friends were aware of it. In spite of failing health, he was able to carry out to the end the duties of his first academic year, completing the work of the Summer term, though with some difficulty. During the vacation he became seriously ill, and, after some fluctuations, the end came on September 4, 1915, the immediate cause of death being a rapid onset of tuberculosis. His friend Lang, who was with him almost at the last, says: ‘He was very weak and changed, but, when he talked, absolutely himself. It was the bravest end to a long fight.’

I saw him last myself, about the end of January, when he was in his usual health, and we had a long and lively talk, partly on botanical subjects, especially that strange fossil, *Tempskya*, and partly on the war.

His position as a botanist, and the great services to science which he was able to render in the course of a too short life, will best be considered after a brief survey of his published work. Before going on to this, a few words may be added about his relations to the British Association and other Societies.

When the Association met at Glasgow in 1901, Gwynne-Vaughan was one of the Secretaries of Section K. He was again Secretary at Winnipeg, Sheffield, and Portsmouth (1909-11), and at Dundee and Birmingham

(1912-13) he was Recorder of the Section. He contributed greatly to the success of the Botanical Section while an officer, and would have made an excellent President if he had lived.

In 1909 he received the MacDougall Brisbane Medal for Research from the Royal Society of Edinburgh, and in 1912 became a Member of the Royal Irish Academy. In 1914 he was appointed External Examiner to the University of Glasgow.

He was elected a Fellow of the Linnean Society in 1907, and was a member of Council at the time of his death.

Published Work.

Gwynne-Vaughan's first publication was a Note in 'Science Gossip' (June 1894) on the rare Crucifer, *Arabis stricta*, Huds., the Bristol Rock Cress. He had found it on an ancient camp, near Llandrindod, Radnorshire, and sent it to the Editors, who confirmed his identification, adding that 'any records of so rare a plant are particularly interesting'. So far as I know, it had previously been recorded only from Clifton and Cheddar, and its speedy extinction was anticipated by Bentham and Hooker.

The first published result of his research work at Kew was a Note in the 'Annals of Botany' (1896. 1), 'On a New Case of Polystely in Dicotyledons'. At that time we were very keen on polystely and such phenomena, under the influence of Van Tieghem's anatomical conceptions. Gwynne-Vaughan showed that the tuber-bearing stolons in certain species of *Nymphaea* have a clearly polystelic structure, which he compared with the structure of the main stem in the related genera *Cabomba* and *Brasenia*. What was still more important in those days, he was able to demonstrate, within the family, a complete series of transitions between polystely and astely.

The paper read at the British Association that year, which so impressed Professor Bower, covered nearly the same ground, but added the new fact that 'root-bearing steles' occur in the rhizomes of *Victoria* and *Nymphaea* spp. (1896. 2).

The full paper 'On the Morphology and Anatomy of the Nymphaeaceae' was published in the Transactions of the Linnean Society (1897. 1). I had to read it for him (on Feb. 18) as he was then busy at Glasgow. It is a rather miscellaneous paper, for he began with the morphology of the leaf, showing how the ontogeny of the individual adult leaf repeats the successive forms of the seedling leaves, constituting a special case of Recapitulation. He also investigated the origin of the peltate form of leaf. He pointed out the remarkable absence of any primitive stages, whether morphological or anatomical, in the seedling of *Nelumbium*, so different from the conditions in the Water-lilies.

The anatomical portion of the paper is an extension of the results communicated in his preliminary notes, illustrated by excellent drawings.

From a purely anatomical point of view, Gwynne-Vaughan's other Kew paper, 'On Polystely in the Genus *Primula*' (1897. 2), is perhaps more important. He paid much attention to the variations of stelar structure in the same species and in different parts of the individual plant. He was able to show that gamostely is not an advanced condition due to the fusion of steles, but is more primitive than dialystely, and that a gamodesmic structure (with the bundles united, but not completed to form steles) probably preceded either. He followed the anatomical development of the seedling in gamostelic and dialystelic species with much care, and found different modes of transition from the original monostely to the perfect or imperfect polystely of the adult stem. His results marked a distinct advance on Van Tieghem's interpretations, and had a direct bearing on his own later work on the Ferns.

This work, as we have seen, was in full progress during the interval between his two tropical journeys. Publication did not begin till some time after his return from the Malayan expedition, when the first paper, on the Anatomy of Solenostelic Ferns appeared, dealing with the remarkable New Zealand Fern, *Loxsonia Cunninghamii* (1901. 1). He revived the term 'solenostely' invented but afterwards discarded by Van Tieghem; in place of the then familiar 'gamostely', on the ground that the latter term implies a *fusion* of steles supposed to be originally distinct; in most cases this is the reverse of the truth, the solenostelic preceding the dialystelic condition, as shown by the development of the individual plant. The conception which lies at the root of Gwynne-Vaughan's Fern anatomy thus follows directly from the results he first attained by his study of the *Primulas*.

The solenostele is defined as a single hollow vascular cylinder, interrupted only by the departure of the leaf-traces. The conception is thus narrower than that of gamostely. In Jeffrey's terminology, Gwynne-Vaughan's solenostely forms 'a special type of amphiphloic phyllosiphony', i. e. a vascular tube with internal as well as external phloem, interrupted by leaf-gaps.

The comparative rarity of solenostely is pointed out, the Ferns offering the chief examples.

The detailed investigation of *Loxsonia* not only established an excellent type of solenostely, but proved interesting in other ways, especially in the demonstration of two kinds of protoxylem, endarch and spiral in the leaf-trace, exarch and scalariform in the stele. The affinity of the genus was regarded as closest with the Dennstaedtiads, while some relation to Gleicheniaceae, Hymenophyllaceae, and Schizaeaceae was also recognized.

The second part of the Solenostelic work, which appeared two years later (1903), had a much wider scope, and extended to Ferns of other than, solenostelic structure. It is, in fact, one of the chief existing contributions to Filicinean anatomy.

Gwynne-Vaughan showed that the dictyostely (typical polystely or dialystely of Van Tieghem), so common in Ferns, is 'primarily due simply and solely to the overlapping of the leaf-gaps in a solenostele', though other gaps in the tube may occur, as in *Dicksonia rubiginosa*. He traced the origin from the solenostele of internal vascular strands, and of internal tubes, such as were found by him in *Pteris elata*, and were previously known in *Matonia*.

Incidentally he called attention to a curious historical point. 'Having first come to the conclusion that Ferns do not possess true seeds, Cesalpino proceeded to deduce the fact that they cannot possess true stems either.'

This, then, was the strange origin of the belief, so obstinately maintained, even down to our own times, that the 'caudex' of a Fern is merely a sympodium of leaf-traces. It also became the source of the 'phyton' theories of Gaudichand and others. Gwynne-Vaughan showed that the apparent segmentation of certain Ferns is really to be regarded as a late development rather than as a primitive feature. In connexion with this question he investigated the course of the internal strands of Cyatheaceae and other Ferns, and found, in agreement with Trécul, that they are not decurrent leaf-traces, but strictly and essentially cauline.

He thoroughly worked out the development of the dictyostele in the young plant of *Alsophila excelsa*, and showed that after the protostelic stage a core of phloem first appears, then the internal pericycle, next the endodermis, and finally the central ground tissue, the structure thus becoming a solenostele. The leaf-gaps as they begin to overlap convert this into a dictyostele, and ultimately the complexity of the mature structure is attained by the nipping off of the internal strands from the main vascular tube.

When lateral shoots are developed they repeat, more or less imperfectly, the ontogeny of the plant as a whole.

The paper, in which I have only indicated a few characteristic points, concluded with a theoretical discussion of the theory of the stele. Gwynne-Vaughan strongly inclined to the view that the origin of the central parenchyma in the higher Ferns was to be regarded as stelar, though he admitted that Jeffrey's opinion, that it arose from the cortex, was theoretically possible.

The diagrams of the stelar structure in the solid are an excellent feature of the plates, and so are the combined photographs and drawings, an under-exposed print being used as a *camera-lucida* sketch.

The paper is altogether an admirable type of modern anatomical work,

under the influence of the Theory of Descent—the comparative anatomy of the Darwinian period at its best.¹

Going back to the year 1901, there is a Note 'On the Nature of the Stele in *Equisetum*' (1901. 2), a subject on which he never published in full, though he continued to give it much attention. He regarded the vascular bundle of *Equisetum* as a compound structure—'of the three strands of xylem present in each bundle of the internode, the carinal strand alone passes out at the node as a leaf-trace'. 'So the xylem of the so-called vascular bundle of *Equisetum* consists of three strands, two of which are lateral and cauline, while the median, or carinal, strand is common to both stem and leaf. The fact that only a small portion passes out as a leaf-trace, and not the bundle as a whole, constitutes an essential point of difference between it and the bundle of a *Phanerogam*.'

He further pointed out that the lateral xylem-strands in *E. giganteum* gave a strong impression of centripetal development. He compared the stele of *Equisetum* with the protostele of *Sphenophyllum*, and with the various degrees of medullation found in the *Lepidodendreae*, and suggested that the lateral xylem-strands in the recent genus 'may perhaps be taken to represent the last remnants of a primitive central mass'. His idea was that the lateral strands in *Equisetum* might correspond to the prominent points of the primary xylem in *Sigillaria*, in the hollows between which the protoxylems lie.

This Note must not be taken as representing Gwynne-Vaughan's final view of the morphology of the stem in *Equisetum*. Judging from letters of his (1912), and from manuscripts he left, it appears that while he always maintained his view of the triple nature of the bundle, and of the essentially protostelic character of the vascular system, he was inclined to give up the centripetal development of the lateral strands, and to admit the possible occurrence of leaf-gaps in certain cases. The whole question is an interesting one, and it is much to be regretted that Gwynne-Vaughan's mature views were never fully recorded.

In a Note (1902) 'On an Unexplained Point in the Anatomy of *Helminthostachys Zeylanica*', Gwynne-Vaughan described for the first time the curious canals which lead down through the cortex almost to the stele, one in front of each leaf. He thought it 'possible that they represent the last indications of vestigial axillary buds', a suggestion which has since been confirmed by the work of his friend Lang.²

We owe to Gwynne-Vaughan (1905. 1) the first description of the anatomy of the new genus of Marattiaceae, *Archangiopteris*, discovered by Dr. Henry in China. The structure was compared with that of *Kaulfussia*,

¹ The British Association Note (1901. 3) is preliminary to the paper just considered.

² W. H. Lang, Studies in the Morphology and Anatomy of the Ophioglossaceae. III, p. 14. Ann. of Bot., January, 1915, vol. xxix.

both being relatively simple forms anatomically. Interesting conclusions were drawn regarding the anatomical distinctions between the Marattiales and Filicales; among the characters peculiar to the former group the internal protophloem is the most striking.

Another paper of the same date (1905, 2) has a curious title: 'On the Possible Existence of a Fern Stem having the Form of a Lattice-work Tube'. The epidermal pockets found in the Ostrich Fern and other species are compared with the *endodermal* pockets which started the lattice-work structure of the vascular system in so many Ferns. If the epidermal pockets likewise became continuous, the whole stem might assume the form of a lattice-work tube. Both series of changes are due to the increasing dominance of the leaf and the leaf-traces. It is an ingenious little paper of an unusual kind.

Leaving the joint series with Kidston for future consideration, we will next take Gwynne-Vaughan's much-disputed work 'On the Real Nature of the Tracheae in the Ferns' (1908). In this paper he endeavoured to show:

(a) That the pits in the Ferns investigated are open.

(b) That the middle lamella of the tracheal wall is absorbed, leaving an open vertical passage between the bars of thickening.

The investigation was suggested by the appearances observed in some of the fossil Osmundaceae. He summed up his conclusions as follows: 'The xylem elements of the Pteridophyta are, for the most part, vessels with true perforations in their longitudinal as well as in their terminal walls.'

'In the Osmundaceae, *Nephrodium Filix-mas*, and probably others, a special type of vessel occurs which is characterized by the complete disappearance of the primary tracheal wall at certain points, so that the cavities of the pits are vertically continuous in the middle of the wall.'

Gwynne-Vaughan's results were controverted by Halft in 1910, and by Miss N. Bancroft in the following year. Their work leads to the conclusion that the old view was right, i.e. that the pits in Pteridophyta are, as a rule, closed, and that the middle lamella is persistent.

Gwynne-Vaughan was in touch with Miss Bancroft's work, and helped her with criticism and advice.

This is not the place to discuss the question, but I may be permitted to quote the following passage from a letter of Mrs. Gwynne-Vaughan's, which defines her husband's position in the matter:

'With regard to the Fern-tracheae I think I can best summarize my husband's view by saying that he thought the question required further investigation. I do not think he would ever have undertaken this himself; it did not interest him as much as the other things he had worked on.'

We have now reached the period of Gwynne-Vaughan's co-operation with Kidston, a most happy conjunction which gave rise to much good

work, and especially to the fine series of memoirs on the Fossil Osmundaceae.

I am told that Kidston and his future colleague first met at Cambridge, during the British Association Meeting of 1904, and at once became friends.

Their joint work began soon after while Gwynne-Vaughan was still at Glasgow, but their first paper was published in 1907, the year he left, and from that time onwards they had to arrange special meetings during vacations, usually at Stirling. A well-known photograph shows them hard at work together in the study at 12 Clarendon Place.

The series on Fossil Osmundaceae is of exceptional interest, for the authors trace back an existing family from recent times to the Permian, on sound evidence, mainly anatomical. Nothing quite like this has been done for any other group. There are five Parts, ranging in date from 1907 to 1914, all published in admirable form in the Transactions of the Royal Society of Edinburgh.

Part I (K. and G.-V., 1907) deals with four species of *Osmundites*: *O. Dunlopi* and *Gibbiana*, new species from the Jurassic of New Zealand, *O. Dowkeri*, Carr., from the Lower Eocene of Herne Bay, and *O. skidegatenensis*, Penh., from the Lower Cretaceous of the Queen Charlotte Islands. The first three are more or less typical members of the family; *O. Dunlopi* approaches *Todea*, but was found to have a 'practically continuous xylem-ring'; the other two are nearer *Osmunda*.

The Pacific species, *O. skidegatenensis*, intermediate in age between the New Zealand and the Herne Bay fossils, showed a much more complex structure than either, or than any living species. The wonderful preservation exhibited the structure to perfection, and the plant proved to have well-developed internal phloem, continuous through the leaf-gaps with the external phloem-zone. In fact *O. skidegatenensis* comes very near the *dictyostelic* ancestor of the Osmundaceae, postulated by Jeffrey. Kidston and Gwynne-Vaughan, however, regarded the plant rather as marking the culminating point in the development of the family.

In this first memoir the authors anticipated the discovery of ancestral types with a continuous zone, or even a solid mass of xylem, and suggested a common origin of the Osmundaceae with *Botryopteris* and *Zygopteris*.

Their anticipation was soon realized; in their second memoir (K. and G.-V., 1908. 1) they described two species from the Upper Permian of Russia, establishing the new genus *Zalesskya* for them. In *Z. gracilis* (Eichwald) they found a wide, continuous ring of wood, divided into two zones, the inner of which consisted of short and broad tracheides. In *Z. diploxylon*, sp. nov., there was evidence that the xylem was solid, the inner zone extending to the centre of the stele. This may also have been the case in the former species, though there the internal tissue had entirely

perished. The rest of the structure, especially that of the petioles, appears to leave no doubt of the relationship to Osmundaceae.

In the third memoir (K. and G.-V., 1909) other species from the Russian Permian are described, of which *Thamnopteris Schlechtendalii* (Eichwald) is much the most important. In this fine fossil conclusive evidence was found for protostelic structure, the inner region of the solid xylem consisting here also of large, thin-walled, reticulate tracheae.

A full account was also given of the structure of the leaf-trace, which passes from mesarchy to endarchy in its outward course. A preliminary communication on this point had been published in the previous year (G.-V. and K., 1908, 2). The origin of the endarch from the mesarch leaf-trace was held to be general in the Filicales.

In the 'general conclusions' of Memoir III the authors laid stress on the approach in the stem of *Thamnopteris* to the structure of *Zygopteris corrugata*, and predicted the discovery of a *Zygopteris* with a solid xylem and central short tracheae; this was almost exactly realized immediately afterwards by Dr. Gordon's discovery of the stem of *Diplolabis*, a Lower Carboniferous *Zygopterid*.

The fourth memoir (K. and G.-V., 1910) is concerned with *Osmundites Kolbei*, Seward, from the Wealden of the Cape, and *O. Schenmuitsensis* (Pettko) from the Miocene of Hungary. The latter is well preserved, but of ordinary structure. In *O. Kolbei*, described externally by Seward in 1907, the pith was much compressed, but the authors found that it contained undoubtedly tracheal elements; they add: 'There is no doubt whatever that the tracheal elements are true and real constituents of the central tissue.' They regarded this tissue, therefore, as a 'mixed pith', essentially similar to that in the stele of *Zygopteris Grayi*, *Z. corrugata*, &c. Another point of interest in *O. Kolbei* was that the leaf-traces pass out without immediately causing an interruption of the xylem-ring, i.e. that the leaf-gap only begins some little distance above the outgoing trace.

In this memoir the geological distribution of all the forms so far described is tabulated.

The general discussion is of great value. The importance of Zenetti's remarkable work on the structure of *Osmunda*, written long before there was any knowledge of the fossil history, is fully recognized. The *Zygopterideae* are discussed at length, in the light of Paul Bertrand's elaborate investigations, and their relation to the Osmundaceae considered. The peculiar *Zygopteridean* frond, so unlike anything in recent Ferns, is regarded as derived from the type common to the Osmundaceae and the Filicales generally.

The possibility is recognized that the Permian genera *Zaleskya* and *Thamnopteris* may eventually require a new Order, though on existing evidence they must be included in the Osmundaceae.

These four memoirs constitute a complete whole, but the observation of additional forms led to the publication, after an interval of four years, of a fifth memoir (K. and G.-V., 1914).

The chief species described are *Osmundites spetsbergensis* (Nathorst) from the Upper Tertiary of Spitzbergen, and *O. Carnieri*, Schuster, a Paraguayan species of doubtful age (Jurassic—Tertiary).

The Spitzbergen species, though it shows no stem, is interesting from the fact that the structure of leaflets and sporangia is preserved, as well as that of the petioles; it is a modern type, resembling *Osmunda Claytoniana* in petiolar structure, and *O. regalis* in the form of the frond. A solenostelic Fern stem was found burrowing among the roots of the *Osmundites*.

O. Carnieri is more remarkable. It is unusually large, the stem measuring 90 mm. and the stele 35 mm. in diameter. The vascular ring is in the form of several meristeles, each surrounded by its own endodermis, and including a varying number of bundles. The preservation is imperfect, but it is probable that the phloem extended all round each meristele, the structure thus being completely dictyostelic. This form is of great interest in conjunction with *O. skidegatisensis*, and may lead to important conclusions when more is known about it.

This completes the main series on Osmundaceae, but there are two small papers by Gwynne-Vaughan alone, dealing with recent members of the same group. The earlier of these, 'Some Remarks on the Anatomy of the Osmundaceae' (1911), is concerned with the possible retention of the primitive features of fossil forms in the young plants of recent Osmundaceae.

The chief definite character found to be retained is the mesarchy of the leaf-trace, occasionally occurring in early leaves of *O. regalis*. The ontogenetic evidence also favours the intrastelar origin of the pith and medullary rays. A full and acute discussion of the general question of Recapitulation is of remarkable interest.

The final paper on the Order is 'On a Mixed Pith in an Anomalous Stem of *Osmunda regalis*' (1914). The abundant medullary tracheae met with in this specimen were evidently formed in response to an injury. The value of the observation depends on the credence attached to Jeffrey's theory that traumatic characters tend to be ancestral. Accepting this, the evidence of the injured specimen tells in favour of the protostelic theory of Osmundaceous anatomy. The argument is a good one, as addressed to the Jeffrey school, but the morphological value of traumatic characters is still very much of an open question.

The Osmundaceae work as a whole is perhaps unique as a study of the comparative anatomy of recent and fossil members of a definite group, ranging over so long a period of geological time. Certain points are still the subject of controversy, and some of the facts may admit of diverse

interpretations, but the value of the work accomplished by Kidston and Gwynne-Vaughan is solid and lasting.

Two more palaeobotanical memoirs by the same authors remain to be noticed. The first of these (K. and G.-V., 1911) gives the first intelligible and satisfactory account of the structure of *Tempskya*, a group of fossils which had puzzled palaeobotanists ever since the year 1824. *Tempskya* consists of a dense mass of Fern roots, traversed by a number of larger organs, variously described as petioles or stems. The authors investigated an unusually good specimen of a *Tempskya* from Russian Turkestan, which they named *T. Rossica*. They found that it consisted of a number of solenostelic Fern stems, running longitudinally through a dense felt of their own adventitious roots. The stems bear two rows of leaves on one side and roots on the other, thus showing themselves to be of dorsiventral structure. They keep parallel to each other and to the general course of the roots, but face indiscriminately in all directions. They branch dichotomously. The authors' interpretation of the facts is that the numerous stems with their felt of roots together formed an erect 'false stem', the individual true stems becoming free at the top and bearing the leaves. A restoration of the plant as it is conceived to have appeared in nature was prepared; it was not published with the paper, but was shown at the British Association Meeting at Sheffield in 1910, and has been reproduced in Dr. Marie C. Stopes's British Museum Catalogue of the Cretaceous Flora, Part II, p. 15.

This interpretation of the structure receives support from the analogy of a recent Tree-fern, *Hemitelia crenulata*, described by Schoute in 1906; in this strange plant a number of branches are actually felted together by a mass of their own roots, to form a false stem.

The excellent paper on *Tempskya*, as it appeared in the Transactions of a Russian Society, is not very accessible to English readers, but a full abstract will be found in Dr. Stopes's Catalogue, above referred to.

The memoir on *Stenomyelon tuedianum*, Kidston (K. and G.-V., 1912), was intended to form the first of a series on the Carboniferous Flora of Berwickshire. So many important forms remain to be described, that it is to be hoped that Dr. Kidston, in spite of the grievous loss of his collaborator, will see the scheme through.

Stenomyelon, originally found by Matheson in 1859, and re-discovered by Kidston, who obtained much better specimens, in 1901, is of Lower Carboniferous age, and is a remarkable and isolated form, referred to the transitional group which we call Cycadofilices. The structure is well preserved, and the investigation does it justice. The memoir is clear, full, concise, and perfectly illustrated, and may well serve as a model of structural work in palaeobotany.

We have now passed in rapid survey through all the published work in which Gwynne-Vaughan took part.

Two papers, I am informed, were left unpublished, but in a sufficiently advanced state to warrant the hope that they may soon see the light. Their subjects are: 'On some climbing Davallias and the Petiole of *Lygodium*', and 'Observations on the Anatomy of the Leaf in the Osmundaceae'.

There is also a considerable amount of manuscript with many Figures, relating to the genus *Equisetum*, in which he had so strong an interest. The chief part of this material was prepared with a view to a joint work on Equisetales and other groups which Gwynne-Vaughan and I had in contemplation. Its fate is still undecided.

Gwynne-Vaughan's chief subject as an investigator may be described as the comparative anatomy of Plants from the point of view of Descent. His work belongs essentially to the Darwinian period; if he had lived, it would have been interesting to see how far he might have been influenced by the modern tendency in the direction of Experimental Morphology. But I think the phylogenetic interest would always have been the main one to him, as it is to the present writer.

He became an anatomist at Kew; it was at Glasgow that he was led to specialize, with such admirable success, on the anatomy of Pteridophyta and above all Ferns. In this subject, so characteristic of modern English Botany, no one surpassed him.

The phylogenetic study of Fern anatomy demands a knowledge of fossil forms, and here his association with Kidston was of the happiest influence, and gave rise to the best of his later work. He made the utmost use of his opportunities, and I was often impressed by his own accurate knowledge of fossil types.

He had accomplished so much, and his judgement was so thoroughly sound, that great things might have been looked for from him, if all had not ended so much too soon.

Gwynne-Vaughan's original work lay within a well-defined field, but he was actively interested in other branches of his science, and, in particular, was very keen on the Algae, at which he worked hard on his occasional visits to the coast, from Glasgow and elsewhere.

With students, who are generally good judges of character, he was, I am told, remarkably successful. At Glasgow, for example, he took a great interest in the athletic life of the University, and was very popular. In all his various posts he established a tradition among his students of trying to obtain first-hand information and to demonstrate things for themselves. Both in the laboratory and on excursions he had the power of inspiring interest and of making difficulties clear.

Those who knew him only from his published work, necessarily of a technical character, would have formed little idea of him as he really was.

Like Darwin, he was a sportsman even before he was a naturalist; we have seen how it was he who did the shooting on the Malay expedition; he always kept his taste for open-air pleasures.

Though so much of his life was spent at Universities, he was very far from having an academic mind; his love for Natural Science was inborn. This is shown by the way he came to Kew, entirely of his own choice, as soon as he was free to follow his natural bent.

Another side of his character is shown by his marked interest in Malayan demonology, and generally in the customs and sayings of a primitive people. Here, again, his interest was sympathetic, and not merely that of an anthropologist.

Gwynne-Vaughan's personality was altogether attractive and original, and those who knew him felt that he was somehow different from the usual type of scientific man of his generation.

He was a delightful and amusing companion, while at the same time his manner had a pleasant touch of old-world courtesy. He made warm friends, as was natural. Now that he has gone, so prematurely, we feel that, whether personally or scientifically, there could scarcely have been a greater loss to English Botany.

D. H. SCOTT.

LIST OF PUBLISHED RESEARCHES.

1896. 1. On a New Case of Polystely in Dicotyledons. Note. *Annals of Botany*, 1896.
1896. 2. On some Points in the Morphology and Anatomy of the Nymphaeaceae. *Annals of Botany*, 1896.
1897. 1. On the Morphology and Anatomy of the Nymphaeaceae. *Trans. Linn. Soc. Lond.*, 1897.
1897. 2. On Polystely in the genus *Primula*. *Annals of Botany*, 1897.
- Observations on the Anatomy of Solenostelic Ferns:
 1901. 1. Part I.—*Loxsona*. *Annals of Botany*, 1901.
 1903. Part II. *Annals of Botany*, 1903.
1901. 2. Remarks on the Nature of the Stele of *Equisetum*. *Annals of Botany*, 1901.
1901. 3. Some Observations upon the Vascular Anatomy of the Cyatheaceae. *Annals of Botany*, 1901.
1902. On an Unexplained Point in the Anatomy of *Helminthostachys Zeylanica*. *Annals of Botany*, 1902.
1905. 1. On the Anatomy of *Archangiopteris Henryi* and of other Marattiaceae. *Annals of Botany*, 1905.
1905. 2. On the Possible Existence of a Fern Stem having the Form of a Lattice-work Tube. *New Phytologist*, 1905.
1908. On the Real Nature of the Tracheae in the Ferns. *Annals of Botany*, 1908.
1911. Some Remarks on the Anatomy of the Osmundaceae. *Annals of Botany*, 1911.
1914. On a Mixed Pith in an anomalous Stem of *Osmunda regalis*. *Annals of Botany*, 1914.

In collaboration with R. Kidston, Esq., LL.D., F.R.S., F.G.S.

On the Fossil Osmundaceae:

- KIDSTON and GWYNNE-VAUGHAN. 1907. Part I.—*Osmundites*, sp. novae. Trans. Roy. Soc. Edin., 1907.
- " " 1908. 1. Part II.—*Zaleskya*. Ibid., 1908.
- " " 1909. Part III.—*Thannopteris*, *Bathypteris*, *Anomorrhoea*. Ibid., 1909.
- " " 1910. Part IV.—*Osmundites Kolbei*. Zygopteridene. Ibid., 1910.
- " " 1914. Part V.—*Osmundites* spp. Ibid., 1914.
- GWYNNE-VAUGHAN and KIDSTON. 1908. 2: On the Origin of the Adaxially Curved Leaf-trace in the Filicales. Proc. Roy. Soc. Edin., 1908.
- KIDSTON and GWYNNE-VAUGHAN. 1911. On a New Species of *Tempskya* from Russia. Verhandlungen der Russ. Kais. Mineral. Gesellschaft, Bd. xlviii, 1911.
- " " 1912. On the Carboniferous Flora of Berwickshire. Part I. *Stenomyelon tuedianum*. Trans. Roy. Soc. Edin., 1912.

The portrait is from a photograph taken in 1911 by Frederick Hollyer, 9 Pembroke Square, Kensington, W.

Some Points in the Morphology of the Stipules in the Stellatae, with special reference to *Galium*.

BY

H. TAKEDA, D.I.C.

With twenty-seven Figures in the Text.



IT is hardly necessary to recapitulate here the results of the previous investigators on the nature of the leaf-like organs or 'leaves' in the apparent whorls of *Galium*, and of many other members of the Stellatae (or Galieae, a tribe of the Rubiaceae). There is little doubt that in any whorl the two opposite 'leaves', one at any rate of which subtends an axillary shoot, are the true leaves, while the other members at the same node are stipules. Thus, in the case of a six-membered whorl there are two leaves, each of which is provided with two stipules. Where only five or four 'leaves' occur in a whorl, it is usually understood that in the first case one, and in the second case both, pairs of stipules have undergone a concrescence. If, however, more than six 'leaves' are present in a whorl, it is explained that one or more of the original four stipules have undergone chorisis, resulting in the production of supernumerary members.¹

Eichler found in *Galium Mollugo* and also *Rubia tinctorum*, that there are often two primordia which fuse, giving rise to a single interfoliar stipule on either side of the stem.² Goebel,³ on the other hand, found in

¹ Cf. de Candolle, *Vegetable Organography* (Engl. ed.), vol. i (1841), p. 286; Le Maout et Decaisne, *Traité général de Botanique*, p. 15 (1868); Leunis, *Synopsis der Pflanzenkunde*, ed. 3, i, p. 193 (1883); Pax, *Allgemeine Morphologie der Pflanzen*, p. 102 (1890); Velenovsky, *Vergleichende Morphologie der Pflanzen*, pt. 2, p. 433 et seq. (1907); Worsdel, *Principles of Plant-Teratology*, vol. i (1915), p. 172. It may be of some interest to mention that Wydler, who recorded the occurrence of a complete fission of a stipule into two separate organs in *Galium Cruciatum*, Linn. (in *Flora*, vol. xlii, 1859, p. 10), suggested that each of the apparently single stipules in this species may be the product of a fusion of two separate organs. 'If so,' he says, 'the formation of a midrib (indistinguishable from the midrib of the true leaves) on the common border of two fused stipules is remarkable. The midrib in the (fused) stipules would then correspond to commissural ribs, like those for example in a gamosepalous calyx.'

² Eichler, *Entwicklungsgeschichte des Blattes*, &c., p. 32, Taf. i, Fig. 18 (1861). Pax (l. c.) follows this view, and gives *Galium rotundifolium* and *G. palustre* as examples.

³ *Vergleichende Entwicklungsgeschichte der Pflanzenorgane*. Schenk's *Handbuch der Botanik*, iii (1884), pt. 1, Fig. 48 B, p. 231. See also Goebel, *Organography of Plants* (Engl. ed.), pt. 2 (1905), p. 369.

Galium palustre that the concrescence of two stipular primordia, as described by Eichler, is of very rare occurrence, and that, as a rule, only a single primordium gives rise to a single stipule.

Franke,¹ who had fresh material of a good many species of *Galium* and other genera of the Stellatae at his disposal, has confirmed Goebel's observations, and states² that the four-membered whorls in the Stellatae arise without exception from four uniform protuberances. He reached the conclusion by investigation of *Galium aetnium*, *G. boreale*, *G. Cruciatum*, *G. lucidum*, *G. Mollugo*, *G. parisiense*, *G. physocarpum*, *G. pusillum*, *G. recurvum*, *G. rubioides*, *G. saccharatum*, *G. sylvestre*, *G. tenuissimum*, *G. verum*, and several other members of the Stellatae, including *Rubia tinctorum*.

It seems worth while, therefore, to inquire how far the feature described by Franke is general, and also whether there is any further evidence for Eichler's statement.

Penzig³ records that in *Rubia peregrina* one often finds whorls in which two forked stipules occur opposite one another, or, where the fission is complete, that six 'leaves' (i. e. two leaves and the two pairs of stipules belonging to them) are present at a node. He also mentions⁴ that in *Galium Cruciatum* the stipules are occasionally partially or completely divided, so that whorls with one or two of the laminae forked, or with five to six separate members, arise.

The writer can add *Galium gracile*, Bunge,⁵ as giving an example of a similar phenomenon. This species, which is widely distributed over Japan and China, constantly possesses four 'leaves' at each node, two being the true leaves and the other two stipular. Although no specimen has ever been found with more than four 'leaves' at a node, examples are however fairly frequent in which one or both of the stipules of a whorl have two midribs, indicating their double nature. The apex of the stipules with double midribs is usually more or less indented, but cases are occasionally found in which the apex is almost entire. There also occur, though less frequently, stipules with a forked midrib. Figs. 1-8 have been selected from two specimens of this plant gathered by Swinhoe in the interior of Amoy, China, to illustrate these features. Fig. 1 represents an ordinary stipule which possesses a single midrib and two lateral veins, and assumes exactly the same shape and size as the true leaf. Fig. 2 is

¹ Beiträge zur Morphologie und Entwicklungsgeschichte der Stellaten. Botanische Zeitung, vol. liv (1890), pt. 1, p. 33 et seq.

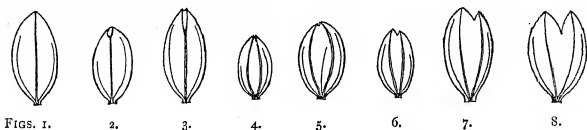
² Franke, l. c., p. 50.

³ Pflanzen-Teratologie, systematisch geordnet, vol. ii (1894), p. 37.

⁴ Penzig, l. c., p. 38.

⁵ Enumeratio plantarum quas in China boreali collegit (1832), p. 35. For a more detailed description see Makino in Tōkyō Botanical Magazine, vol. xvii (1904), p. 74. The plant has also been described as *G. miltorhizum*: Hance, in Seemann's Journal of Botany, vol. vi (1868), p. 114.

a similar stipule, but the midrib is forked at the tip, obviously indicating the fused nature of the stipule. Fig. 3 shows the midrib still more deeply forked, the apex of the stipule being at the same time slightly indented. Fig. 4 has two complete midribs as well as the two lateral veins which are normally present, while the apex of the stipule is very shallowly indented. This is the type of the double stipule which commonly occurs in this species. Fig. 5 shows a shallow notch at the apex, while one of the two midribs is provided with two lateral veins; one of the latter being in the middle region of the stipule. Figs. 6 and 7 are stipules with a deeper notch at the apex and exemplify a type of much less common occurrence. Fig. 8 shows the deepest cleft the writer has seen.



Figs. 1-8. Stipules of *Galium gracile*, Bunge. All $\times 1.5$. Explanation in the text.

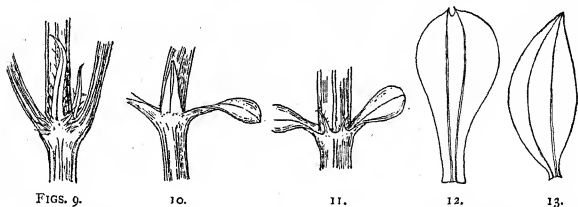
It is obvious that the appearance of a double midrib is not due to an abnormally strong development of one of the lateral veins, since the lateral veins are always present in the stipules, whether they have one or two midribs. Moreover, the midrib may sometimes fork into two, as shown in Figs. 2 and 3, and this phenomenon is held to be a step towards the production of two complete midribs. The stipule with two midribs and three lateral veins (Fig. 5) represents the nearest approach observed to a separation of the stipule into two complete organs. Since the development of the leaves and stipules could not be investigated in the material at the writer's disposal, it is impossible to state definitely whether the ordinary stipules (i. e. those with a single midrib) are always produced as the result of a true concrescence of two primordia. It is quite possible that each of the ordinary stipules is usually produced from a single primordium, and that two types of development may be found in one and the same species.

Another instance is afforded by *Galium paradoxum*, Maxim.,¹ which also is a Far Eastern species.² This plant possesses four 'leaves' at each

¹ Bulletin de l'Acad. Imp. de St.-Petersb., vol. xix (1873), p. 281.

² This species was first discovered in Manchuria in 1860. Later, in 1879, Franchet and Savatier (Enum. Pl. Japon., vol. ii, p. 392) recorded it from Japan. Its occurrence in China (Hupeh) was first made known by Diels (Engler's Bot. Jahrb., vol. xix, 1901, p. 583), and in Korea by Komarov (Flora Manchuriae, vol. ii, 1907, p. 497). It is not uncommon in the mountainous districts of Japan, but is apparently scarce in China. From the latter country the writer has seen the following specimens: Hupeh, Chienshih (Henry, No. 5851), Patung (Henry, No. 6026), Changyang (Wilson, No. 1153), Yunnan; ad collem Yen-tze-hay, alt. 3,200 m. (Delavay, No. 3102).

node in the middle and upper parts of the stem, i. e. two true leaves and two stipules, the latter resembling the former, but easily distinguishable from the true leaves at the same node by the fact that they are often smaller, and also that they are always bearded at the base of the petiole. In the lower part of the stem there are again two interfoliar stipules, which, however, are quite distinct in appearance from the true leaves, being small and scale-like. They are subulate or triangular, membranous, often thinly ciliate on the margin, and also more or less barbate at the base (Fig. 9). In one of the specimens collected on Mt. Fuji in 1887, and sent to Kew from the Tōkyō Imperial University, the writer found a small, scale-like stipule with a forked midrib at a node in the lowermost region of the stem, the apex of the stipule being at the same time slightly indented (Fig. 10). The writer has not been so fortunate as to find in this species



FIGS. 9-11. *Galium paradoxum*, Maxim., showing scale-like interfoliar stipules from the lower region of stem. Figs. 9 and 11, $\times 7$, fig. 10 $\times 6$. Fig. 12. *Galium saxatile*, Linn. Stipule with two separate midribs. $\times 6$. Fig. 13. *Asperula asterocephala*, Bornm. Stipule with two separate midribs. $\times 2$.

any stipules with two separate midribs. A case has, however, been found in which two separate, small, scale-like stipules are present on one side of a node near the very base of the stem of one of the specimens gathered by Wilson in Changyang, in the province of Hupeh, China (Fig. 11).

A third instance of 'four-leaved' species occasionally producing stipules with two midribs is presented by *Asperula asterocephala*, Bornm.¹ This handsome perennial, which was discovered by Bornmüller in Kurdistan, attains a height of about 40 cm., and always bears four 'leaves' at each node, just as the first example given above. The stipules can readily be distinguished from the true leaves by the fact that they are always distinctly smaller than the latter at the same node, and one-nerved, while the true leaves are often provided with one or two lateral veins on either side of the midrib. Towards the apex of the stem the stipules become very much reduced in size, often to such an extent as to appear almost scale-like,

¹ In Mithell. Thür. Bot. Ver., N. Folge, vol. vii (1895), p. 7.

although they are always green and not scarious, thus differing from the scale-like stipules at the lower nodes in *Galium paradoxum*, Maxim. In one of the co-type specimens of *A. asterocephala*, which was distributed under J. Bornmüller, Iter Persico-turcicum, No. 1337, the writer found at the third node from the base a stipule having two midribs, while its general appearance is very similar, except for size, to the true leaves at the same node (Fig. 13). So far as has been seen, the occurrence of stipules of this nature seems to be rare in this species.

As another instance, *Galium leiophyllum*, Boiss. et Hohenack.,¹ in which the writer found a stipule of a double nature, may be briefly described below. This plant is of moderate size² and bears five, or more often six 'leaves' to a node. In one of the specimens distributed under J. Bornmüller, Iter Persicum alterum, No. 7105,³ the writer found below the middle region of a stem (about the fifth node from the base) a whorl consisting of two true leaves and two stipules, one of the latter being furnished with two midribs (Fig. 21). The node immediately above and that below this whorl bear five 'leaves' each, and all the nodes in the higher region of the same stem are six-membered.

The writer also found in a specimen of *Galium saxatile*, Linn., which he collected himself in England in 1915, a stipule with two midribs. It occurred in a four-membered whorl which was preceded by a five-membered whorl, and succeeded by a six-membered one (Fig. 12).

Further cases of the occurrence of stipules of a double nature in four-membered whorls have been found in *Asperula arvensis*, Linn. (Fig. 19), *A. sherardioides* (Boiss.), Jaub. et Spach. (Fig. 20), and *A. aspera* (M. Bieb.), Boiss., to each of which further allusion will be made later on.

It may, therefore, be not unreasonable to conclude from the instances above referred to, that in certain species of *Galium* and some other members of the Stellatae, such as those which were investigated by Goebel and by Franke, each stipule in a four-membered whorl arises from a single primordium, while in some species such as *G. gracile*, *G. paradoxum*, *G. leiophyllum*, and *G. Cruciata*, and the three species of *Asperula* above referred to, stipules are occasionally produced as the result of a coalescence of two primordia. The first case is regarded as a congenital concrescence,⁴ while the second is a true concrescence, and at the same time points towards the production of more than two stipules at a node.

In regard to the whorl with five members, mention has already been made above of the result of the investigation carried out by Eichler,⁵ who found in *Galium Mollugo* that two stipular primordia on one side of a node

¹ Boissier, Diagnoses, vol. i, 3 (1843) p. 36; Ejusd. Fl. Orient., vol. iii. (1875), p. 51.

² 30-45 cm. in height.

³ This specimen is to be referred to var. *subvelutinum*: Boiss., l. c.

⁴ Goebel, ll. cc.

⁵ l. c., p. 32, Taf. i, Fig. 15.

fuse, and thus give rise to a single stipule, while each of the two stipular primordia on the other side of the same node develops into a separate organ.

Franke,¹ on the other hand, states that in this case the solitary stipule on one side of the node is produced from a primordium which is homologous with a stipular primordium of the four-membered whorl, while the paired stipules on the other side of the node arise from a common arc-like protuberance, which is from the beginning distinguishable by shape and insertion. The latter phenomenon is, however, not to be considered as a fission in the proper sense. In other words, each of the three stipules in the five-membered whorl arises from a single primordium, and there is no evidence of a true concrescence of two stipular primordia giving rise to a single organ.

So far as the writer's own observations go, stipules of a double nature are very rarely met with in five-membered whorls. The only plant in which such an occurrence has been found is *Asperula aspera* (M. Bieb.), Boiss. (Fig. 22). This case, however, gives sufficient evidence for the conclusion that stipules are occasionally produced also in five-membered whorls as the result of a true concrescence of two primordia. A further reference to this plant will be made later on with regard to the distribution of double stipules.

As to the case in which more than six members occur in a whorl, it is regarded that in addition to the two true leaves there are more than two stipules on one or both sides of the stem. Eichler² found in *Galium Mollugo* that a new tissue arises between the original two stipular primordia and produces an independent organ resembling the other stipules. If this phenomenon takes place on one side only of a stem, there would be five stipules produced. If, however, a new tissue between two primordia occurs on both sides of the stem, there would be six stipules formed, thus making the whorl eight-membered at a node.

Franke³ has confirmed Eichler's observations, and states that he found in *Asperula odorata* and *A. azurea* a new primordium arising between two stipular primordia on one or both sides of the node, the former being distinguishable from the latter by its smaller size.

Neither of these investigators has examined any whorl with more than eight members, although there are certain species of *Galium*, such as *G. verum*, Linn., and of some other genera, e. g. *Asperula odorata*, Linn., which often bear more than eight, and, particularly in the former plant, up to twelve, foliar members (i. e. two true leaves and ten stipules) at a node.

Unfortunately, the writer's own observations on these pleiomorous whorls in herbarium material cannot throw any light upon the subject. It may, however, be presumed that there arise as many primordia as there

¹ l. c., p. 50.

² l. c., p. 32, Taf. i, Figs. 16, 17.

³ l. c., p. 51, Taf. i, Figs. 7, 8.

are 'leaves' at a node. Thus, as many as five stipular primordia may occur on either side of a node.

In this connexion mention may be made of some interesting features exhibited by *Didymaea mexicana*, Hook. fil.,¹ which also belongs to the Stellatae. As a unique character² of this (monotypic) genus the plant has been described as having each of its (opposite) leaves provided with a pair of typically differentiated stipules, the latter being subulate or lanceolate and quite distinct from the true leaves.³

In *Didymaea* there are as a rule four scale-like stipules at a node,⁴ but very often three stipules are present on one or on both sides of the stem, and the middle or additional one is then distinctly smaller than either of the other two (Figs. 14, 15).⁵ This feature reminds one of the primordial stage of the seven- or eight-membered whorls in *Asperula*.⁶ Although nothing is known about the development of the foliar organs in *Didymaea*, it

¹ In Benth. et Hook., *Genera Plantarum*, vol. ii (1873), p. 150; Hooker's *Icones Plantarum*, vol. xiii (1878), p. 55, tab. 1271.

² That is, unique among the Stellatae.

³ K. Schumann gives in Engler and Prantl's *Pflanzenfamilien*, vol. iv (1891), pt. 4, p. 3, *Rubia diphylla*, K. Schum., as another example of a member of the Stellatae having ordinary triangular stipules. Neither the figure he refers to nor a description of this plant has ever been given. There is, however, little room for doubt that this name (nom. nud.) is a synonym of *Relbunium diphyllum*, K. Schum. (apud Martius, *Flora Brasil.*, vol. vi, pt. 6, 1888, p. 117; also see under this genus in the *Pflanzenfamilien*, vol. iv, p. 154). This plant possesses a minute triangular interfoliar stipule on either side of the node, just as in *Rubia ephedroides*, Cham. et Schltdl. (*Linnaea*, vol. lli, 1828, p. 231; also see Martius, *Fl. Brasil.*, vol. vi, pt. 6, p. 120, Tab. xciii, Fig. 1), and *R. equisetoides*, Cham. et Schltdl. (l. c., p. 231; Martius, l. c., p. 119). These three plants are, however, apparently xerophytes and have their foliar organs very much reduced; the true leaves being scale-like, three or four mm. in length, and only a little larger than the stipules, which are similar in shape. It is possible that if the true leaves could be induced to develop to a respectable size, the stipules would also become much larger, assuming exactly the same shape as the former. It is therefore obvious that these three species are not suitable as examples of the exceptional phenomenon among the Stellatae of scale-like stipules alternating with the true leaves, which are decussate and of normal appearance. So far as the writer knows, *Galium paradoxum*, Maxim., *G. geminifolium*, F. von Müller, *Asperula geminifolia*, F. von Müller, and *Didymaea mexicana*, Hook. f., are the only members of the Stellatae showing this rare phenomenon, and of these the first-named plant produces scale-like stipules at the lower nodes only, those at the middle and upper nodes of the stem being leaf-like. Both *Galium geminifolium* and *Asperula geminifolia* are Australian plants (cf. Benth., *Flora Australiensis*, vol. iii, 1866, pp. 445 and 443), and usually bear at each node two opposite, narrow leaves, and two small scale-like stipules alternating with the former (cf. K. Schumann, in Engler and Prantl, *Pflanzenfamilien*, vol. iv, pt. 4, p. 151, Fig. 48 D). In *G. geminifolium*, however, the stipules are occasionally well developed, either becoming leaf-like or showing a transition. This is probably due to a change in some physiological conditions (such as might be caused by rain, &c.) at the time when the foliar organs are developing.

It may be remarked here that Velenovsky (l. c., p. 434) gives *Putoria* as an example of the genera which belong to the Stellatae and bear interpetiolar stipules alternating with two opposite leaves. However, all the leading systematists agree to refer this genus to the tribe Anthospermeae!

⁴ Cf. K. Schumann, in Engler and Prantl, *Pflanzenfamilien*, vol. iv, pt. 4, p. 147, Fig. 47, N. O.

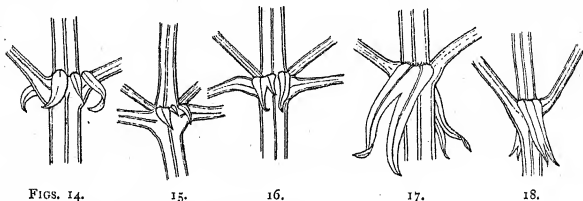
⁵ Only once has the writer met with four stipules on one side of a node; this was in one of the specimens distributed under Pringle, *Plantae mexicanae*, No. 4716 (1894). In this case also, the two middle ones are smaller than the other two stipules.

⁶ Cf. Franke, l. c., p. 51, Taf. i, Fig. 8.



may be presumed that each of the three stipules arises from an independent primordium. The middle stipule, as a rule, shows no definite connexion with either of the adjacent stipules. The writer has, however, found a case in which one of the two stipules on one side of a node is divided about half-way into two unequal parts (Fig. 16),¹ thus suggesting how three stipules may have arisen from two by fission of one of them, that is, if two be taken as representing the fundamental number of stipules on each side of the node.

A feature of further interest has also been found in several specimens examined of *Didymaea*, the paired stipules on either side of the stem being not uncommonly united into a single organ, with the lamina either deeply or shallowly divided (Figs. 17, 18). There is, of course,



FIGS. 14-18. Interfoliar stipules of *Didymaea mexicana*, Hook. fil. All $\times 5$. (Figs. 15 and 16 were taken from inflorescence, the others from stem.) Explanation in the text.

no shadow of doubt that these single stipules with a forked lamina are produced as the result of a concrescence of two separate primordia. The question then arises whether the presence of a single stipule on either side of the stem should be considered as the more primitive type, and if this were so, whether the occurrence of two to three separate stipules on each side of the node might be regarded as the result of a fission of the original single interfoliar stipules.

A comparison of the different features found in *Galium* and other genera with those just described above for *Didymaea* will bring forward the more general question: whether the six-membered whorl in the *Stellatae* should be regarded as having been derived from a four-membered whorl, owing to chorisis of an original pair of opposite interfoliar stipules, or whether, on the other hand, the production of only two stipules at a node is due to reduction.

An examination of the seedlings may throw some light on the problem, since early stages in ontogeny often show some ancestral characters.²

¹ Unfortunately, the material was unsuitable for determining whether the two midribs of this stipule are completely separate or united near the base of the organ in question.

² It is not maintained that characters observed in early stages of ontogeny are necessarily to be

According to Lubbock¹ the seedlings of *Galium saccharatum* and *G. tenuissimum*, both of which usually bear more than six 'leaves' at each node, produce only four 'leaves' (i. e. two true leaves and two stipules) at the first node, five 'leaves' at the second, and often six 'leaves' (i. e. the two true leaves and four stipules) at the third node. The same author states² that the seedling of *Sherardia arvensis* possesses only a single stipule on each side of the stem at the first three nodes, and that four stipules (two on each side) occur at the succeeding nodes. Velenovsky³ states that in the seedling of *Asperula odorata* the cotyledons are succeeded by a four-membered whorl, in which two stipules (smaller) are distinctly enveloped by two opposite leaves. In the next whorl there are six 'leaves', and the two opposite leaves again surround the four stipules, which are produced by a fission of those organs which correspond to the original two opposite stipules at the first node. He further states⁴ that similar features may be seen also in *Galium sylvaticum*, the seedlings of which however produce four-membered whorls throughout the first year's growth.

The writer's own observations on seedlings have revealed more instances of the same phenomenon in other members of the Stellatae. *Galium murale*, All.,⁵ always produces four 'leaves' at each of the lower nodes, and five or six 'leaves' at the upper nodes. Both *G. setaceum*, Lam.,⁶ and *Crucianella disticha*, Boiss.,⁷ bear four leaves at the first, and often up to the third node, and six 'leaves' at each of the higher nodes. *G. Vaillantii*, DC.,⁸ which possesses eight 'leaves' at each node in the upper region of the stem, produces four 'leaves' at the first, and sometimes also at the second node, but five or more 'leaves' at each of the nodes at a slightly higher level. *G. Aparine*, Linn., usually produces four 'leaves' at the first, and sometimes the second node, and less commonly five or six 'leaves' at the first node. In *Crucianella angustifolia*, Linn., and *C. patula*, Linn., the first and often the second and third nodes have a whorl of four members, while each of the higher nodes is furnished with a six-membered whorl. *C. latifolia*, Linn., which is another 'six-leaved' species, bears four

accepted as ancestral (cf. Lang, Address to the Botanical Section, Brit. Ass., at Manchester, 1915, p. 6). Any given case, relating for instance to the leaves of a seedling, must be studied in the light of as much collateral evidence as is available, and a slight presumption in favour of ancestral character may be granted on the strength of a number of examples, such as the seedlings of the gorse, certain phyllode-bearing acacias, many Conifers (cf. Veitch's Manual of the Coniferae, ed. 2, p. 22 et seq., 1900), &c.

¹ Seedlings, vol. ii (1892), p. 77.

² Lubbock, l. c., p. 79.

³ l. c., pp. 434-5.

⁴ Velenovsky, l. c., p. 435.

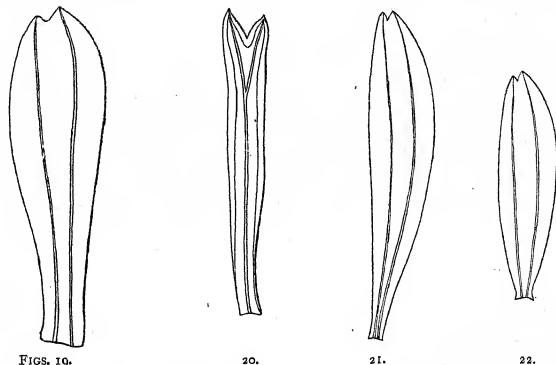
⁵ Fl. Pedemont, i, p. 8, Tab. 77, Fig. 1 (1785); a Mediterranean species.

⁶ Encyclopédie, vol. ii (1806), p. 584; a Mediterranean and temperate Asiatic species.

⁷ Diagnoses, vol. i, pt. 3 (1843), p. 25.

⁸ Flore de France, no. 3381 (1805); Prodrômus, vol. iv (1830), p. 608. The plant is sometimes treated as a variety of *G. Aparine*, Linn., var. *Vaillantii*, Koch, or a subspecies of *G. Aparine*, Linn.: see Hooker's Stud. Fl., ed. 3 (1884), p. 194.

'leaves' at each of the first few nodes, or often for several nodes. *Meri-carpaea vaillantoides*, Boiss.,¹ a small annual plant from Assyria, is described by some authors as having four 'leaves' at each node,² and by others six 'leaves'.³ This discrepancy is due to the fact that the plant actually bears four 'leaves' at each of the lower nodes, and six 'leaves' to the node in the upper region of the stem. A highly interesting feature is furnished by a specimen of *Asperula arvensis*, Linn., which usually produces four 'leaves' at the first node, and five or six members at the second. The specimen under consideration is one of those distributed under Siehe's Botanische Reise nach Cilicien, No. 144. One of the two stipules at the first node



FIGS. 19. Double stipule of *Asperula arvensis*, Linn. $\times 5$. FIG. 20. Forked stipule of *Asperula sherardioides*, Jaub. et Spach. (seen from under surface, showing forked midrib and recurved margin of lamina). $\times 5$. FIG. 21. Double stipule of *Galium leiophyllum*, Boiss. et Hohenack. $\times 5$. FIG. 22. Ditto of *Asperula aspera*, Boiss. $\times 5$.

in this particular specimen is provided with two midribs and a notched lamina (Fig. 19), evidently showing a transition to the five-membered whorl at the next node. A specimen of *Asperula sherardioides* (Boiss.), Jaub. et Spach.,⁴ has also been found to exhibit a similar phenomenon. This Persian plant is a small annual and does not attain more than 10 cm. in height. The first node usually possesses four, but very rarely six 'leaves', while the second node has five or six leaves, or occasionally four.

¹ Diagnoses, vol. i, pt. 3 (1843), p. 52. Cf. Hook. f. in Benth. et Hook., Gen. Fl., vol. ii (1873), p. 149.

² Cf. Hook. f. in Benth. et Hook., Gen. Fl., vol. ii (1873), p. 149.

³ Cf. Boissier, Fl. Orient., vol. iii (1875), p. 83.

⁴ Illustr. Pl. Orient., vol. i (1843) p. 153, tab. 83; Boissier, Fl. Orient., vol. iii (1875), p. 29. The plant was originally described under the name of *Crucianella sherardioides*, Boiss.: Diagnoses, vol. i, pt. 3 (1843), p. 24.

The particular specimen referred to above was distributed under No. 7093 of J. Bornmüller, Iter Persicum alterum. It measures about 4 cm. high, and possesses four 'leaves' at each of the first two nodes, while the third node, which is situated immediately below the inflorescence, has six 'leaves'. One of the two stipules of the second node has the lamina and midrib forked (Fig. 20), while the other is normal.

It is therefore evident that, so far as our knowledge goes, in *Galium* and in some allied genera, the species with several to many 'leaves' at each node generally start in the seedling with a four-membered whorl, which is succeeded by whorls consisting of a larger number of 'leaves'.

Correlating these facts, it appears probable that in the *Stellatae* the four-membered whorl (composed of two true leaves and an interfoliar stipule on each side of the stem) represents the more primitive type, while the whorls with more than four members (i.e. with more than two stipules) represent a derived type.

In connexion with the arrangement of 'leaves' in the seedlings, it may be of no little interest to examine the manner in which double stipules (i.e. those with a forked or double midrib) are distributed on the stem of adult plants, and their relation to the 'leaves' at the neighbouring nodes. Among the three 'four-leaved' species of *Galium* above mentioned, *G. gracile*, in which double stipules are frequently met with, produces the stipules of this nature generally in the middle and upper regions, and only rarely near the base of the stem. If a double stipule is present at a node, it is often found that a few succeeding nodes also bear one or two stipules of the same character. In some cases, double stipules may be found at several nodes on a stem, while in the more usual cases such stipules are produced only at one or two nodes. *Asperula trifida*, Makino,¹ furnishes us with a more interesting case. This rare species is a perennial, and occurs in certain mountainous districts of Japan. The plant was described as having 'leaves four—rarely five—verticillate', but it occasionally produces as many as six 'leaves' at a node. The specimens examined were gathered on Mount Ishidzuchi, in the Province of Iyo, in August, 1888, and were sent to Kew from the Tōkyō Imperial University. They bear four, more usually five, 'leaves' at each of the lower nodes, and often six 'leaves' to the node in the middle region of the stem (vide *infra*). Towards the upper part of the stem, a six-membered whorl is as a rule suddenly succeeded by four-membered whorls. Those whorls which are situated in close proximity to the inflorescence have the stipules much reduced in size.² The arrangement of the 'leaves' in one of the specimens

¹ Illustr. Fl. Japan, vol. i, No. 11 (1891), p. 2, tab. 68; and in Tōkyō Bot. Mag., vol. xvii (1903), p. 72.

² See the excellent figures by Makino, l.c. This feature is often met with in the *Stellatae*. It is especially noticeable in such a species as *Asperula asterocephala*, Bornm., above referred to.

examined shows the following sequence: the first node of the aerial stem, which is continuous with a rhizome, has four 'leaves', the second five, the third four (?),¹ the fourth five, the fifth four, the sixth four (?),¹ the seventh to ninth six each, the tenth four 'leaves', one of the two stipules having a forked midrib (Fig. 23); the eleventh again bears four 'leaves', and one of the stipules, which are smaller than the true leaves at the same node, has a slight indication of a double nature; the twelfth, which is giving off a branch terminated with an inflorescence, has four members with the two stipules markedly smaller than the true leaves at the same node; the thirteenth whorl, which is at the ultimate node on the stem, consists of two true leaves (7 to 8 mm. long), and a small but normal stipule (4 mm. long) on one side, and two minute stipules (2 mm. long) on the other side of the node. From this node three peduncles have sprung, two of which have at their first (and only) node two small true leaves only, stipules being completely suppressed, while the remaining peduncle bears at its lowest node a pair of true leaves and a minute stipule (1 mm. long) on one side of the stem. Another specimen examined shows a somewhat similar feature: at the first and second nodes the 'leaves' have withered and are torn off; the third node, which is very similar in appearance to the fifth node of the first specimen above described, has five 'leaves'; the fourth node bears four 'leaves', and one of the stipules is equal to the true leaves, while the other is broader and is provided with two midribs (Fig. 24); the fifth to seventh nodes are all six-membered; the eighth node has four 'leaves', and one of the stipules of this whorl possesses a forked midrib, and is exactly the same in appearance as that delineated in Fig. 22, while the other is normal; the ninth node is again four-membered but without any double stipule; the tenth node is similar in every respect to the eighth node, with this difference, that the midrib of the double stipule is more deeply forked (Fig. 25); the eleventh (ultimate) node is also four-membered, with two stipules considerably smaller than the true leaves. At the ultimate node of another specimen the writer found two true leaves² and two stipules, the latter being much reduced in size, and one of them being provided with two complete midribs (Fig. 26).³

As has already been mentioned above, the occurrence of double stipules in the case of nodes with more than four 'leaves' is very rare.⁴ The only instance the writer has found is presented by *Asperula aspera*

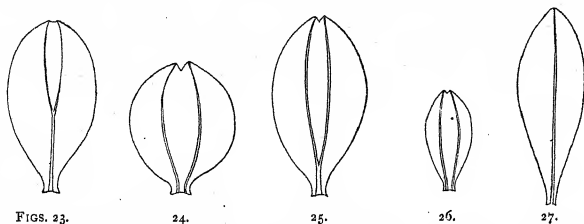
¹ The whorl has been damaged, and consequently it is very difficult to determine this point with absolute accuracy.

² One of these leaves is shown in Fig. 27 for comparison.

³ Since this species bears flowers with a usually three-, often four-, and rarely five-lobed corolla, a search was made with a view to detect corolla-lobes with a forked vein. Unfortunately, the result has so far been negative. A propos, it may be mentioned that *Galium saxatile*, Linn., also produces pentamerous flowers very frequently.

⁴ No case in which a double stipule occurs in a whorl with more than five 'leaves' has been observed or recorded.

(M. Bieb.), Boiss.¹ This plant, which is a native of the Caucasus and Persia, usually has six 'leaves' to a node. In the upper region of the stem of one of the specimens collected by Szovits in Persia and sent to Kew from the Imperial Botanic Garden in Petrograd, the writer found a whorl consisting of two true leaves and three stipules, one of which is provided with two midribs, the lamina being at the same time very shallowly notched at the apex. Unfortunately, the specimen is unsuitable for examining the number of 'leaves' at the next nodes above and below the one just mentioned. In this plant, branches are given off from several of the lower nodes. The branches usually start with nodes which bear four 'leaves' to each, and are gradually succeeded by five- and six-membered



FIGS. 23.

24.

25.

26.

27.

Figs. 23-27. *Asperula trifida*, Makino. 23-26. Double stipules. 27. True leaf from the node at which the double stipule delineated in Fig. 26 is borne. All $\times 5$.

whorls, as the branches become elongated.² In the same series of specimens the writer found two cases in which one of the stipules of a five-membered whorl had two midribs. The arrangement of the 'leaves' on one of the branches is as follows: the first node has two true leaves and two stipules, one of the latter having two midribs; the second node is four-membered, both of the stipules being normal; the third node five-membered, one of the stipules again having a double midrib (Fig. 22); while the fourth whorl is just sprouting, thus preventing the determination of the number of 'leaves' with accuracy.

The examples described above clearly show that double stipules may occur in any part, but more often near the base, and also towards the apex of a stem. It may also be remarked that double stipules fall on the whole into two categories, according as they form a transition towards increase or decrease in the number of 'leaves' in a whorl. In other words,

¹ Fl. Orient., vol. iii (1875), p. 28. The plant was originally described as *Crucianella aspera*, M. Bieb.: Fl. Taunico-Caucasica, vol. i (1808), p. 107.

² A similar feature is also found in *G. Aparine*, Linn. In the seedling of this species, and also of others belonging to the Stellatae, branches are very frequently produced in the axil of the cotyledons.

double stipules may be produced in the region in which the number of 'leaves' is undergoing increase, or on the other hand reduction from node to node. It is evident that at the base of a seedling a double stipule represents a stage of progression, while towards the apex of a stem it usually represents a stage of retrogression. It has already been pointed out that in the *Stellatae* the four-membered whorl seems to represent within the limit of possibility the most primitive type. Hence it follows that the progression generally speaking would have been from the four-membered whorls to those with six or more members by fission of the original two stipules. In the case of any 'four-leaved' species, such as *Galium gracile*, the occurrence of double stipules therefore indicates a step towards the production of 'five-leaved' species.

A case presenting some phenomena similar to those described above (though not involving stipules) has been recorded by Groom,¹ who found in *Lysimachia vulgaris* that dimerous nodes occurred at the base, tri- to tetramerous nodes higher up on the stem, and finally in the uppermost region of the stem dimerous nodes reappeared. In this case the transition was often accomplished by one or two double leaves at intermediate nodes. The same author² found in *Rhinanthus Crista-galli* that a double leaf stood at the transitional region from cyclic arrangement of leaves to acyclic. It appears certain that in the former case (i.e. *Lysimachia*) the double leaves represent on the whole in the lower region of the stem a stage of progression, and in the upper region one of retrogression. In the latter case (i.e. *Rhinanthus*) the double leaf doubtless represents a stage in the complete replacement of two opposite leaves by a solitary leaf, by means of a concrescence.³

In dealing with double stipules (and double leaves), it should be borne in mind that the foliar organs of this nature may sometimes be produced without relation to the general tendency of arrangement and distribution of 'leaves' in the stem. Some of the cases above described may again be referred to. In the case of *Galium leiophyllum* a four-membered whorl near the base of a stem is succeeded by a five-membered whorl, thus showing a tendency of increase in the number of 'leaves'. At the next node the whorl is again four-membered, but one of the two stipules has two midribs (Fig. 21), apparently indicating a transition towards decrease in number. The next whorl is however again five-membered, and all the nodes (about four have so far developed) succeeding this particular one are six-membered. In the examples of *Asperula trifida*, which have been described above in detail, the number of members at succeeding nodes does not show a regular

¹ Longitudinal Symmetry in Phanerogamia. Phil. Trans. Roy. Soc. Lond., B, vol. cc (1908), p. 84 et seq.

² Groom, l. c., p. 106.

³ For further instances of similar phenomena see Worsdell, Principles of Plant-Teratology, vol. i (1915), p. 216 et seq.

sequence, but repetition of increase and decrease may be seen. In such cases, double stipules may be clearly associated with either increase or decrease, or sometimes a decision on the subject may be impossible.¹ At the same time it may be noticed in many cases that in alternate nodes the number of the 'leaves' and also the nature of the stipules are more closely correlated than in successive ones.

Explanations may be sought for partly in some physiological factors which either promote or retard the growth of a plant. Boodle² found in the seedlings of the gorse that similar irregularities often occurred regarding the distribution of the trifoliate leaves. He is of opinion that this is at any rate partly due to variation in some physiological conditions during the growth of the seedlings. On the other hand, Groom's suggestion³ regarding a similar phenomenon exhibited by *Lysimachia vulgaris*, that 'morphogenetic impulses are transmitted along the orthostichies', may give the explanation of the alternating number of members at succeeding nodes described in some of the above examples.

Turning to the case of a seven- or eight-membered whorl, it may be held that the middle stipule of the three (on one or both sides of the node) has originated by fission from one of the two adjoining stipules. Since the middle stipule usually takes exactly the median position and assumes the same size as the others, its relationship to the one or the other of the two adjoining stipular members cannot be determined. This occurrence may conveniently be termed a congenital fission (chorisis, dédoublement) in contrast with the phenomenon known as congenital fusion. If this congenital fission once starts in any species it may be repeated more than once, resulting in the production of as many stipules as space will permit on either side of the node. Hence, we more often find the pleiomerous whorls in the species with narrow 'leaves' and a comparatively thick stem, such as *Galium verum*.

In the case of the pleiomerous whorls, stipules are known to occur as many as five on either 'side, and they probably develop from as many primordia as there are stipules, without showing any connexion with one another.

A parallel case may be found at the cotyledonary node in many of the Conifers and a few Dicotyledons, in which the two original cotyledons have undergone chorisis, thus giving rise to a polycotyledonous condition. In these cases the increase in the number takes place as a rule without any transitional stages, and double cotyledons are not frequently in evidence. The double cotyledons recorded by Groom⁴ for *Acer Pseudoplatanus*⁵ and

¹ Cf. Worsdell, l. c., p. 238.

² On the Trifoliate and other Leaves of the Gorse (*Ulex europaea*, L.). Ann. Bot., vol. xxviii, (1914), p. 527 et seq.

³ Groom, l. c., p. 86.

⁴ l. c., pp. 102-3.

⁵ Also see Worsdell, l. c., p. 215, Pl. XIX, Fig. 4 a.

Fraxinus excelsior, by Miss Chick¹ for *Torreya Myristica*, and by Hill and de Fraine² for *Cupressus torulosa*, *Abies sibirica*, *Pinus montana* var. *gallica*, *P. contorta* var. *Murrayana*, *Araucaria Cunninghamii*, &c., are therefore worthy of notice.

From the evidence given above, the conjecture may be justified that the direct ancestors of the Stellatae possibly had two stipules at each node, and that each one of these two stipules had been derived by means of congenital concrescence from two separate stipular organs, the earlier ancestors of the Rubiaceae as a whole being assumed to have possessed four stipules at each node. Hence, it follows that the species of *Galium* (and also of any other genera of the Stellatae) having four-membered whorls would on the whole represent the most primitive type in that particular tribe. Whorls with six foliar organs would thus be regarded as a more advanced type among the Stellatae, but at the same time as representing a reversion to the condition found in the ancestors of the Rubiaceae. It is suggestive that among living Rubiaceae cases of concrescence of stipules occur very frequently, and in certain genera, such as *Palicourea*, *Cephaelis*, &c., some species have four separate stipules, while others have two either partially or completely fused (connate) ones, thus resulting in the production of four-membered whorls.

Regarding the stage of evolution at which the stipules assumed the characters of true leaves in the Stellatae, it is difficult to come to a decision. However, it is beyond all doubt that the original type of the stipules in the Stellatae was scale-like, and that the leaf-like stipules have evolved from that type, probably in relation to certain physiological necessities. It may therefore be considered that in this respect *Aidymaea mexicana* stands nearest the prototype of Stellatae, as Goebel maintains,³ and that *Galium paradoxum* represents the most primitive species of its genus. As to the question whether *Galium geminifolium* and *Asperula geminifolia*, both of which usually bear two scale-like interfoliar stipules at each node,⁴ furnish us with further examples of primitive species is rather doubtful. The production of scale-like stipules in these two species may probably have been brought about by the circumstances of their xerophytic habitat, thus indicating reduction rather than a retention of the primitive state.

SUMMARY.

1. In *Galium* and other allied genera, each stipule as a rule develops from a single primordium.
2. Fairly frequently, and particularly in four-membered and rarely in

¹ The Seedling of *Torreya Myristica*. New Phytologist, vol. ii (1903), p. 85.

² Seedling Structure of Gymnosperms, II. Ann. Bot., vol. xxiii (1909), p. 221, Pl. XV, Fig. 4 b.

³ Organography of Plants (Engl. ed.), pt. 2 (1905), p. 371.

⁴ Cf. the present paper, p. 203, foot-note.

five-membered whorls, stipules may be found which have been produced as the result of a coalescence of two primordia. Stipules of this kind (=double stipules) possess either a forked midrib or two separate midribs, the apex of the stipules being at the same time more or less two-lobed.

3. Double stipules may occur near the base, or towards the apex, and more rarely in the middle region of a stem. They may in certain cases represent a transition towards increase in number, in other cases a stage leading towards numerical decrease of the organs. Double stipules forming examples of both of these cases are occasionally found on one and the same stem. Sometimes, however, a decision on this subject is hardly possible.

4. In the seedlings of several species examined of the genera *Galium*, *Asperula*, *Crucianella*, and *Mericanpaca*, the node or sometimes a few nodes succeeding the cotyledonary node as a rule bear a four-membered whorl, consisting of two true (opposite) leaves and two stipules alternating with the former. In the higher region of the stem the number of members in a whorl may in some of the species examined be gradually increased up to eight.

5. The four-membered whorl is considered to represent the primitive type, at the same time indicating the probable character which prevailed among the direct ancestors of the Stellatae.

6. The six-membered whorl, which probably represents the type that characterized the ancestors of the Rubiaceae, is in the Stellatae regarded as having been derived from a four-membered whorl by complete fission (dédoublement) of the two stipules into four.

7. Whorls with more than six members have no doubt originated by repeated fission of the original two stipules.

8. *Didymaea mexicana*, Hook. fil., which bears two opposite leaves, and from two to often six, or rarely seven, scale-like stipules at each node, is presumed to approach the prototype of the Stellatae. And in this species also the four-membered whorl very probably represents the most primitive type.

9. *Galium paradoxum*, Maxim., which bears two leaves and two scale-like stipules at the lower nodes and two true leaves and two leaf-like stipules in the higher region of the stem, is believed to be the most primitive species of the genus in this respect.

The present investigation has been carried out in the Herbarium, Royal Botanic Gardens, Kew. All the specimens, except those of *Galium saxatile* and *G. Aparine*, used for the investigation are preserved in the Herbarium. The writer has great pleasure in expressing his sincere thanks to Sir David Prain, C. M. G., C. I. E., for the privilege of working in the Herbarium and also using the Library. The writer also takes this opportunity of thanking Mr. L. A. Boodle for the constant encouragement, valuable help, and the interest he has taken during the progress of the investigation.

POSTSCRIPT.

After the above had been written, some of the seedlings of several members of the *Stellatae* raised in a hot-pit in the Royal Botanic Gardens, Kew, were found ready for an investigation. The result of an examination regarding the number of members at lower nodes in the stem is as follows:

1. *Galium Aparine*, Linn. The seedlings bore a four-membered whorl at the first node succeeding the cotyledons. Only one specimen was found to have produced a five-membered whorl at the first node. Thus, the result corresponds to what has been observed in the field, as described in the foregoing pages.

2. *Galium Mollugo*, Linn. \times *G. verum*, Linn. So far, the seedlings have produced three nodes, at each of which four 'leaves', consisting of two true leaves and two stipules, are present. The stipules are as a rule similar to the true leaves, but a scale-like stipule occasionally stood opposite a normal, leaf-like stipule.

3. *Asperula galioides*, Bieb.¹ So far, four nodes have developed in the seedlings examined. Four 'leaves' were present at each of the four nodes. The stipules are sometimes smaller than the true leaves in the same whorl.

4. *Asperula tinctoria*, Linn.² So far, three nodes have been produced, each of which is four-membered. The stipules are distinctly smaller than the true leaves at the same node, often assuming a scale-like appearance.

¹ According to de Candolle (*Prodr. Reg. Veg.*, vol. iv, p. 585) this species bears six to eight 'leaves' to the node.

² According to de Candolle (*l. c.*, p. 582) the whorls in the lower region of the stem are six-membered, those in the middle region are four-membered, while those in the apical region are two-membered.

Studies in Seed Germination.

The Genus *Marah* (Megarrhiza), Cucurbitaceae.

BY

ARTHUR W. HILL, M.A., F.L.S.,

Assistant Director, Royal Gardens, Kew.

With Plate V and two Figures in the Text.

SOME dry prickly fruits, which appeared to belong to a species of *Echinocystis*, were received at Kew in 1908 from Mr. F. R. S. Balfour of Dawyck, having been collected by him in the arid Sierra Nevada region of California. The seeds, which were unlike those of any known species of *Echinocystis*, quickly germinated and were found to exhibit a type of germination similar to that of the Californian 'Big root', *Megarrhiza californica*, described and figured by Asa Gray¹ and Darwin.² A further supply of seeds of this plant and also seeds of allied species from California were obtained through the kindness of Mr. Balfour and other correspondents, and an examination of their mode of germination has yielded some results of interest.

In order to ascertain the botanical identity of the seeds originally received, the specimens of *Echinocystis* and allied genera were obtained on loan from the Smithsonian Institution and examined by Mr. S. T. Dunn.³

The seeds were found to belong to the genus *Marah*, Kellog (*Megarrhiza* (Torr.), S. Wats.), which is morphologically quite distinct from *Echinocystis*, Coignaux.

In this remarkable genus the underground tubers are said often to reach the size of a man's body.⁴

It is of interest to find that the systematic grounds on which *Marah* was separated from *Echinocystis* are supported and strengthened by morphological considerations, since not only is the underground tuber a feature peculiar to *Marah*, but the type of germination is essentially geophilous,

¹ Asa Gray : American Journal of Science, vol. xiv, 1877, pp. 21-4, and the Botanical Text Book, Pt. I, Structural Botany, ed. 6, 1879, pp. 20, 21.

² Darwin, C. : The Power of Movements in Plants. 1880, pp. 81-3.

³ Dunn, S. T. : The Genus *Marah*, in Kew Bulletin, 1913, pp. 145-53, and p. 238.

⁴ Hall, H. M., in Univ. Calif. Publ. Botany, vol. i, 1902, p. 19.

resembling that of some Monocotyledons, while in *Echinocystis* the cotyledons are epigeal and the germination of the seed is of the usual cucurbitaceous type.

Dunn, in his revision of the genus *Marah*, enumerates eleven species, and seeds of five of these have been received at Kew.¹ The general type of germination appears to be similar throughout the genus, though differences in detail may occur.

The least specialized type of germination has been found to occur in *M. fabaceus*, and *M. horridus*, the species first examined at Kew, is the most highly developed, whilst *M. macrocarpus*, *M. muricatus*, and *M. micranthus* more nearly approach the condition shown by *M. horridus*.

The plant figured by Asa Gray and Darwin under the name *Megarhiza californica* is probably a seedling of *Marah macrocarpus*, Greene, though the herbarium specimens referred to *Megarhiza californica*, Torr. and S. Wats., have been found to belong to *Marah fabaceus*, Greene.

Asa Gray² was the first to observe and describe the peculiar germination of the seeds of *Megarhiza californica*, and subsequently Darwin³ made them the subject of careful study, and added several important details to the description given by Gray. Darwin's account is detailed and exhaustive, and the only excuse for the present contribution lies in the fact that the germination of the seeds of other species has been studied and has rendered possible a comparative account of seed germination in the genus.

Marah fabaceus, Greene.—In this species the two cotyledons are fused together towards the base and never leave the seed, and their petioles are united to form a short tube. On germination, the petiole tube elongates, and the plumule and radicle are carried out of the seed. The tube is only about 6 mm. long, but on emerging from the micropyle it bends downwards, and the radicle then breaks through the tip and penetrates deeply into the soil, leaving the outer portions of the end of the petiole tube as a frayed edge, much in the same way as occurs with the hypocotyl of the Radish (*Raphanus*) (Pl. V, Fig. 1).

When the root has become well established by sending out lateral roots, the plumule breaks through the petiolar tube opposite the point of emergence of the root, and in time appears above the surface of the soil. At the same time a hypocotyledonary tuber begins to be formed by the re-storage of part of the reserve materials contained in the fleshy cotyledons (Fig. 2). This underground tuber increases in size as a result

¹ Seeds were kindly sent by Mr. H. F. Shorting, Huntington Beach, California, Professor H. M. Hall, College of Agriculture, Berkeley, California, Mr. W. W. Whitney, San Diego, California, Dr. J. N. Rose, Smithsonian Institution, Washington, and Mr. F. R. S. Balfour of Dawyck.

² Amer. Journ. Science, vol. xiv (1877), pp. 21-24, with Figs., and the Botanical Text Book, ed. 6, Pt. I, Structural Botany (1879), pp. 20, 21, Figs. 43, 44.

³ Darwin: Power of Movement in Plants, 1880, pp. 81-3, and Fig. 58 A.

of the metabolic activity of the plumule and radicle, and eventually assumes very large proportions.

Darwin describes the occurrence of absorbent hairs on the petiolar tube of *Megarrhiza californica*, and they have been noticed in all those species with a well-developed cotyledonary tube, but in *Marah fabaceus* no such absorbent hairs are present. This is no doubt due to the shortness of the tube and to the rapidity with which the root bearing its own root-hairs develops. Except for the fact that the petioles of the cotyledons are fused to form a short tube, the type of germination exhibited by this species may be compared with that of *Aesculus*, where a certain elongation of the cotyledonary petioles takes place to carry the radicle and plumule out of the seed.

M. micranthus and *M. macrocarpus*.—The germination of the seeds of *M. muricatus*, Greene, and *M. micranthus*, Dunn, is so similar to that of *M. macrocarpus* (*Megarrhiza californica*) that there is little to add to Darwin's account.

In *M. micranthus* and *M. macrocarpus* the seeds are orbicular or ovoid, about 1.5 cm. long by 6 to 8 mm. broad, and the cotyledons are united towards the base. The petiolar tube in *M. micranthus* reached a length of 2 to 3 cm., and split for some distance on the emergence of the plumule. The hypocotyledonary tuber was ovoid, and quickly emitted roots.

In *M. macrocarpus* the petiole tube grows as much as 6 cm. vertically into the ground, and it is probable that in its native home it may attain a greater length and carry the plumule deep into the soil as a protection against drought (Pl. V, Fig. 9).

Under artificial greenhouse conditions the plumule bursts through the tube soon after the root has become well established, but under natural conditions it is probable that there may be a considerable resting period after the plumule has been safely deposited deep down in the earth, whilst the tuber is developed at the expense of the reserves stored in the seed, and until conditions become favourable for the development of the climbing shoot.

M. muricatus.—A number of seeds of *M. muricatus* were received from California, but only one germinated. In this species, the seed is flattened and somewhat orbicular-rhomboid in outline, 1.8 to 2.0 cm. in diameter. The cotyledons are only 1 to 2 mm. thick, and free except, as in other species, near the base, where they are definitely fused together. A long petiolar tube furnished with absorbent hairs is formed, and the plumule eventually escapes owing to the splitting apart of the component members of the tube at its base. The tuber in this species appears to be partly epicotyledonary in origin at the commencement, though no doubt later the hypocotyl shares in its development. Owing to lack of material, the tuber development could not be followed.

M. horridus.—The species whose germination has been most carefully studied is *M. horridus*, Dunn, seeds of which were collected in the Kaweah River Valley, California, at 6,000 ft., by Mr. F. R. S. Balfour. Of the region it inhabits, Mr. Balfour writes: 'The district for five or six months of the year is under very deep snow, but from May to October there is cloudless sunshine without a drop of rain.' Nothing is known of the germination of the seed under natural conditions, but it is probable that the seed remains buried under the snow, and may be carried into the ground during the winter, germination taking place in the late spring. Judging from observations made under greenhouse conditions, germination when started would proceed rapidly, and the plumular shoot would no doubt soon appear above ground. This shoot, however, in its first year would probably only have a comparatively short existence.

The seeds in *M. horridus* are ovoid with a thick testa, and measure about 3.75 cm. long. At the broad end they are about 2 cm. in diameter across the cotyledons, and at the narrow end towards the apex the diameter is about 1.8 cm. The cotyledons are thick and fleshy, somewhat hollowed and fused together at the base. Each cotyledon is about 4 mm. thick, and is stored with aleurone and oil.

On germination, the testa bursts by a longitudinal fissure commencing at the micropyle and gradually extending along the pronounced ridge encircling the seed, until by the swelling of the cotyledons it is split into two portions. The petiolar tube carrying the plumule and radicle at its apex quickly elongates and bends over, growing vertically downwards into the soil (Pl. V, Fig. 3). The tube is covered with a mat of fine, somewhat woolly, unicellular hairs, to which particles of soil adhere after the manner of root-hairs; these hairs in fact, until the radicle develops from the end of the petiolar tube, apparently perform the function of root-hairs and absorb the moisture required for the development of the embryo (Figs. 3-7).

The tip of the petiolar tube ends abruptly, in the early stages of germination, in a short brown conical tip which is surrounded by a loose and more or less detached flange or root-cap. The flange may in part represent some portion of the inner seed-coat carried away by the tip of the petiolar tube, and is also no doubt part of the cap of the radicle itself whose tip forms the actual apex of the cotyledonary tube (Pl. V, Figs. 3, 4, and 6). While the petioles are elongating and carrying the plumule and radicle into the ground, very little development of either organ takes place (Fig. 5), but after an appropriate depth has been reached the radicle begins to elongate, develops root-hairs, and quickly grows down into the soil and sends out lateral roots, anchoring the plumule firmly in the ground (Pl. V, Fig. 7).

About this time a slight swelling becomes noticeable at the point

where the root and the tube of the petioles are united, and is found to indicate the position of the plumule and the commencing tuber. With the development of the root, the petiolar absorbing hairs dry up and cease to function. The tube of the petioles in the specimen grown at Kew was from 5 to 7.5 cm. in length, but under natural conditions it would no doubt penetrate to a greater depth in the soil. The plumule now commences to elongate and breaks through the petiolar tube near the base, the tube splitting under the pressure into its component halves. The splitting apart of the petioles extends gradually upwards towards the cotyledons, until at length only a small portion of the tube is left intact (cf. Fig. 8).

The apex of the plumule is sharply bent over at the tip, and this strong curvature of the shoot apex is maintained by the mature shoot and may perhaps be attributed to the fact that the plant is a scrambling climber over and among bushes.

With the growth of the plumule and its appearance above ground, accompanied by the production of green leaves, the tuber begins to increase in size, and in particular to broaden at the apex. As a result the cotyledon petioles each become split into three portions which, owing to the continued expansion of the tuber, gradually get separated somewhat widely apart, and the young tuber when dug up is seen to be suspended from the seed by six separate strands, the split petioles of the two cotyledons (Pl. V, Fig. 9).

The base of each strand is swollen out and passes gradually into the tuber, which in some cases may appear to be composed of six semi-independent portions. The young tuber has also been found to be deeply cleft into two from below, the two halves roughly corresponding to the two cotyledons and having been supplied by the materials stored in each (cf. Fig. 9).

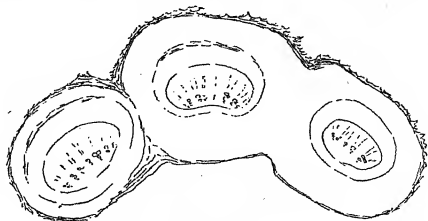
Within the bases of the cotyledon petioles, the tuber develops a somewhat flattened-oval area with the shoot rising from the midst, and on this flattened surface several dormant buds are developed, which no doubt would give rise to aerial shoots should occasion require.

INTERNAL STRUCTURE OF PETIOLES.

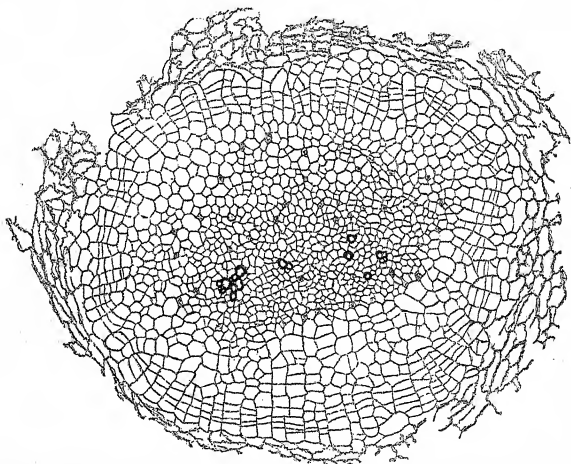
An examination of the petiole tube in transverse section shows usually six vascular bundles, three belonging to each petiole, and if the section be of a petiole tube which has already split into its component portions it is noticeable that the cotyledon petiole shows grooves which correspond to the spaces between the vascular bundles (Text-fig. 1). As the tuber increases in size, these grooves are seen to be the planes of weakness along which the petiole will split into strands, as already described, so that each strand will contain a single vascular bundle.

It is of interest to notice that owing to the splitting apart of the petiole tube into its component halves, the edges formerly in union must, when free, be furnished with a 'false' epidermis. No new 'false' epidermis,

however, appears to be formed on the splitting up of the petioles into strands, but apparently a kind of absciss layer of suberized tissue is laid down, along which the separation takes place. With the friction of the



TEXT-FIG. 1. *Marah horridus*. Petiole or one cotyledon from the split portion of the petiole tube in transverse section, showing the commencement of the further splitting into three strands, each of which will have a vascular bundle. The outer cortical tissues have been worn away.



TEXT-FIG. 2. *M. horridus*. Unit strand of split petiole with active pericycle.

soil and increasing age of the seedling, the outer tissues of the petioles cease to function, and become somewhat disorganized and form an irregular layer of suberized cells.

When the petioles have become split into their several strands, it is found that the vascular bundle of each is surrounded by a band of pericyclic tissue of recent formation, and in an active state of division (Text-fig. 2). This pericyclic periderm gives rise to suberized tissue on the outer side and cortical tissue on its inner side, and the line of communication between the seed and the young plant is thus maintained for a considerable time during the seedling's development.

The vascular bundle contains a small number of lignified elements, but consists mainly of small sieve-tubes and phloem parenchyma.

SUMMARY.

1. On the germination of the seed in *Marah*, the petioles, which are fused together to form a tube, grow out and carry the plumule and radicle into the ground.

2. In *M. fabaceus* the tube is very short and the germination is almost normal, but in *M. horridus* and other species the petiole tube elongates considerably and is furnished with absorbent hairs.

3. The radicle breaks through at the base, and later the plumule penetrates the side of the petiole tube and grows above the soil. Eventually a hypocotyledonary tuber is formed, which may become very large.

4. In *M. horridus* the petiole tube first splits into its two component halves and then, owing to the growth of the tuber, into six separate strands, each of which is furnished with a vascular bundle.

EXPLANATION OF PLATE V.

Figs. 1 and 2, *Marah fabaceus*. Figs. 3-7 and 9, *M. horridus*. Fig. 8, *M. macrocarpus*.

Fig. 1. *Marah fabaceus*. Early stage in the germination of the seed, showing the short petiole tube (ρ), with its tissues frayed and the radicle bent downwards. The plumule has not yet broken through the petiole tube. t , testa; c , cotyledons.

Fig. 2. An older seedling. The plumule (σ) has broken through the tube at the point of curvature of the tube and radicle.

Fig. 3. *M. horridus*. A seed with a portion of the testa removed, showing the petiole tube with a cap (σ) at its apex. The tube measured 1.5 cm. in length and was furnished with a mass of absorbent hairs. (Slightly mag.)

Fig. 4. The flange-like cap (σ) at the apex of the petiole tube. (Mag.)

Fig. 5. The apex of the petiole tube in diagrammatic section, showing the undeveloped plumule (σ), radicle (ρ), and the epidermal hairs.

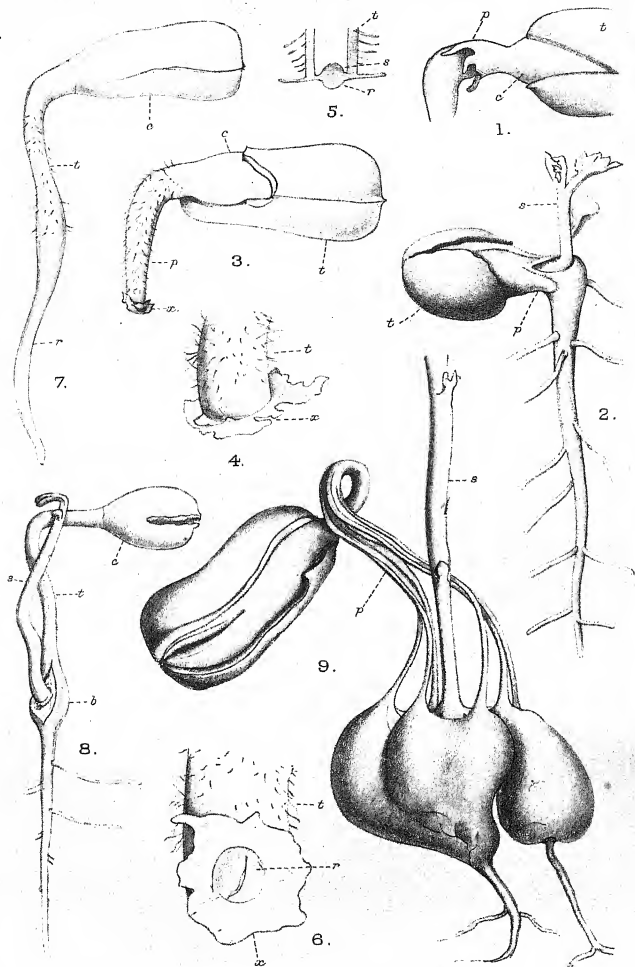
Fig. 6. The apex of the tube, showing the cap. (Mag.)

Fig. 7. An older seedling in which the petiole tube has elongated and the root developed. The point of junction of petiole tube and root is marked by a slight swelling. The testa has been removed. The whole length of tube and root is 7.5 cm., the tube measuring 3.5 cm. (Nat. size.)



Fig. 8. *M. macrocarpus*, Dunn. A seedling in which the plumule (*s.*) has burst through the elongated petiole tube and is pushing up to the surface of the soil with tip bent over. The commencement of the hypocotyledonary tuber (*b.*) is noticeable. The petiole tube is covered by fine hairs. The testa has been removed to show the two partially fused cotyledons (*c.*). (Nat. size.)

Fig. 9. *M. horridus*. An old seedling with well-developed plumular shoot (*s.*) and hypocotyledonary tuber which is still in connexion with the seed by means of the petiole tube. The tube has split first into its two component halves, and each petiole has then split into three strands. The tuber is deeply lobed below and on the upper surface bears adventitious buds. (Nat. size.)



A.W.H. del.

Hush lith. et imp.

A.W. HILL - MARAH.

DEPARTMENT OF BOTANY
UNIVERSITY OF CALIFORNIA

Studies in Permeability.

III. The Absorption of Acids by Plant Tissue.

BY

MILDRED HIND.

With eleven Figures in the Text.

IN the first of these studies it has been shown that cells of potato tuber absorb hydrogen ions from aqueous solutions of hydrochloric acid with considerable rapidity. In this paper an account is given of more detailed experiments made to determine to what extent this rapid absorption is a characteristic of acids in general.

Earlier work (4) appears to indicate that with strong acids it is the concentration of the hydrogen ion which is the determining factor of the action of acid on plants, while with organic acids the anion is stated to influence the action (6). So far, the methods which have been used have given little information as to the extent to which acids are actually absorbed by plant tissue, nor have these methods yielded results of any great accuracy. In the experiments recorded in this paper the methods of physical chemistry advocated and described in previous papers of this series have been used.

Again, so far there has not been produced much evidence as to the substances which are responsible for the absorption of acids. For instance, Czapek (1, 2, 3) seems to be of the opinion that the action of acids on the fat emulsions of the plasma is responsible for the effects produced; on the other hand, Loeb (5, 7), Pauli (11), and Osterhout (9) think that protein substances are active in many of the phenomena of permeability. Some experiments have accordingly been made in the course of this work in regard to the action of acids on various plant substances, but however suggestive the results of these experiments may be, it is of course impossible to draw from a few isolated experiments any definite conclusions in regard to the structure and function of cell membranes.

This work was undertaken at the suggestion of Mr. Stiles and Mr. Jørgensen, whom I would here thank for their advice during its progress.

METHODS.

1. *Electrical Conductivity Methods.*—By measuring the change in electrical conductivity of a solution surrounding plant tissue, some idea of the effect of the solution on plant cells can be obtained. If the substance in solution enters without harming the cell and causing an exosmosis of the cell-contents, a decrease in conductivity will be the result; whereas if the plasma-membrane is affected in such a way that the substances within the cell diffuse out, the conductivity of the external solution will be increased on account of this. The change in conductivity in such cases will therefore be the resultant of the diffusion of the external solute into the cell and of exosmosis of electrolytes from the cell.

These experiments were carried out in the same way as the electrical conductivity experiments described in the first of these studies. Twenty discs of potato tuber were put in a stoppered bottle containing 100 c.c. acid, the conductivity of which had been measured. The conductivity of the acid solution was then taken at intervals. Dilute solutions of each acid in various concentrations were used. The experiments were all done in duplicate; they were carried out at constant temperature—18° C.—as it has already been shown that temperature exerts a considerable influence on the rate of absorption (13).

The curves were obtained by plotting the increase or decrease in electrical conductivity against the time.

2. *Volumetric Analytical Methods.*—In order to measure the acidity of the external solution after it had been in contact with the potato discs for different times, the solutions were poured off and titrated with standard alkali. No reliable results, however, were obtained, owing to the great dilution of the solutions, and possibly to the diffusion out from the cells of substances which interfered with the indicators.

3. *Electrometric Method.*—The method of experimentation involving the use of electrical conductivity can only give a rough approximation to the actual course of absorption of electrolytes, owing to the diffusion out of electrolytes from the cell, which takes place with most substances and even to some extent in distilled water. By measuring the concentration of the hydrogen ions in acid solutions before and after they have been in contact with potato discs, the actual rate of absorption of the hydrogen ion can be measured. Experiments were carried out in the way described in the second paper of this series (13).

The hydrogen-ion concentration was calculated in terms of the original pure acid solution. The curves were obtained by plotting the relative hydrogen-ion concentration against the time. The bottles containing the discs and acid were kept at a temperature of 18° C. throughout the experiments.

This method gives no definite information as to the absorption of the anion. Whether the acid is absorbed as such, or whether the kation and anion enter the cell at different rates, is a question at present unsolved. The work of Pantanelli and Sella (10) indicates the possibility of this latter alternative with its resulting complications.

THE RESULTS.

I. The Absorption of Acids by Potato Tissue.

Hydrochloric Acid.

Series 1.—The rapid absorption by plant cells of the hydrogen ion of hydrochloric acid in a concentration of $\frac{N}{1000}$ has been indicated in previous

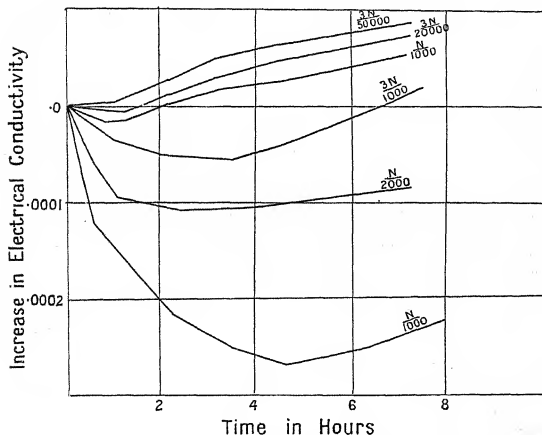


FIG. 1. Potato in Hydrochloric Acid.

papers (12, 13). In the present investigation a wide range of acid concentrations was employed, varying from $\frac{N}{1000}$ to $\frac{3N}{50000}$. The curves shown in Fig. 1 indicate that a marked decrease in the electrical conductivity of the acid solution occurs with all strengths of acid except the very dilute ones. In all cases examined, the conductivity rose after a time. These curves indicate that in all cases a rapid absorption of acid by the potato

cells takes place, but in the case of the lowest concentrations of acid the increase of conductivity due to the electrolytic exudate from the cells more than counterbalances the decrease, owing to removal of hydrogen ions by the potato tissue. This influence of the exudate on the conductivity curves makes itself obvious after a time in the case of the higher strengths of acid.

Series 2.—More definite information as to the absorption of hydro-

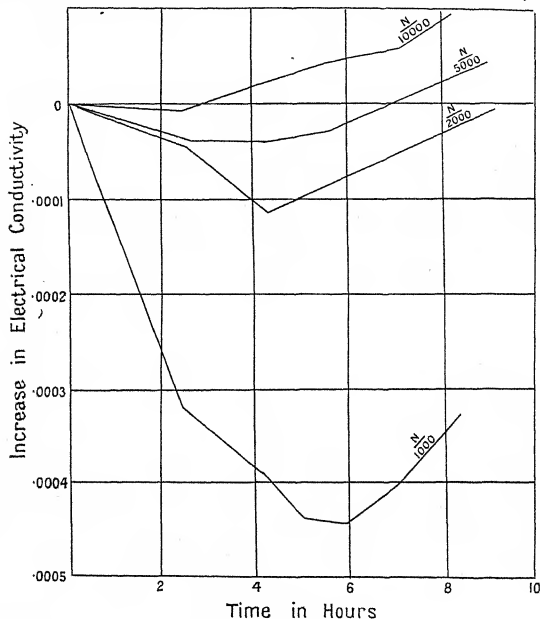


FIG. 2. Potato in Nitric Acid.

chloric acid was obtained by the electrometric measurement of the change in hydrogen-ion concentration of the solution. Experiments were carried out with acid of concentrations $\frac{N}{1000}$, $\frac{N}{2000}$, $\frac{N}{5000}$. In all cases there was a rapid absorption of hydrogen ions, as already indicated for the case of $\frac{N}{1000}$ acid in previous papers of this series.

Nitric Acid.

Series 3.—Conductivity measurements were made of the external solution, as in Series 1, when potato discs were immersed in nitric acid solutions of concentrations $\frac{N}{1000}$, $\frac{N}{2000}$, $\frac{N}{3000}$, $\frac{N}{10000}$.

The accompanying figure (Fig. 2) shows that the result with nitric acid is similar to that obtained with hydrochloric acid.

Series 4.—Experiments made with the electrometric method of measurement, to determine the rate of absorption of the hydrogen ion from

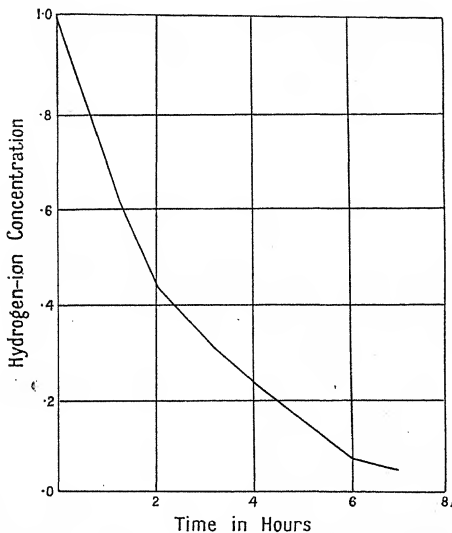


FIG. 3. Potato in $\frac{N}{1000}$ Nitric Acid.

nitric acid solutions, show that the kation is absorbed rapidly, as in the case of hydrochloric acid. The curve shown in Fig. 3 indicates the rate of absorption of the hydrogen ion when $\frac{N}{1000}$ nitric acid was used.

Sulphuric Acid.

Series 5.—Similar experiments were made with sulphuric acid as with hydrochloric and nitric acids. The change in conductivity of the external solution, when solutions of strengths $\frac{N}{500}$, $\frac{N}{1000}$, $\frac{N}{2000}$, $\frac{N}{5000}$, and $\frac{N}{10000}$

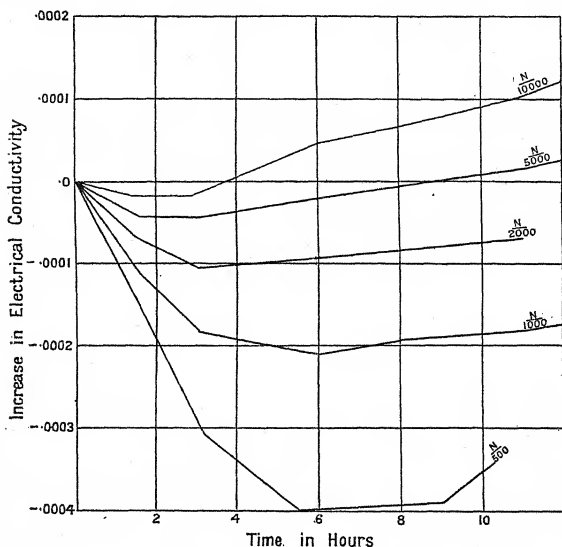


FIG. 4. Potato in Sulphuric Acid.

were used, are shown graphically in Fig. 4. It will be observed that the general results are similar to those given with hydrochloric and nitric acids.

Oxalic Acid.

Series 6.—The absorption of oxalic acid by potato tissue was investigated in the cases of solutions of concentrations $\frac{N}{500}$, $\frac{N}{1000}$, $\frac{N}{5000}$. The change which takes place in the conductivity of the external solution is

similar to that in the case of the strong mineral acids, but as Fig. 5 indicates the decrease in conductivity is less, which may be due to a smaller absorption of hydrogen ions, or to the greater exosmosis of electrolytes.

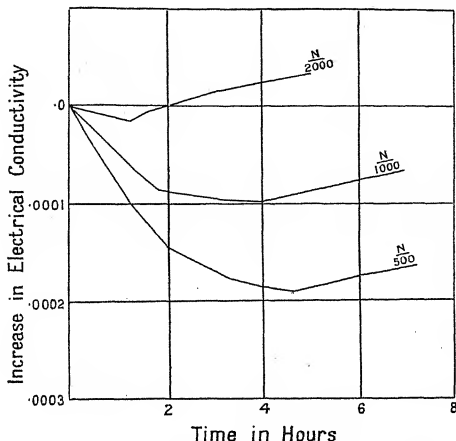


FIG. 5. Potato in Oxalic Acid.

Formic Acid.

Series 7 and 8.—In the case of formic acid considerably different results were obtained. Conductivity measurements of the external solution were made with acid of original concentration $\frac{N}{500}$, $\frac{N}{1000}$, $\frac{N}{2000}$. In all cases the conductivity showed at first a slight decrease, which speedily gave place to a rapid increase, this increase being greater the higher the concentration of the acid (cf. Fig. 6).

Measurements of the actual concentration of the hydrogen ion were made in the case of $\frac{N}{1000}$ acid.

Fig. 7 indicates the course of the absorption. It will be observed that during the first few hours the hydrogen ion is absorbed at almost the same rate as the hydrogen ions of nitric acid. After a time, however, a remarkable change occurs. This consists in the concentration of the hydrogen ion of the external solution increasing, a phenomenon which was never observed in the case of the inorganic acids.

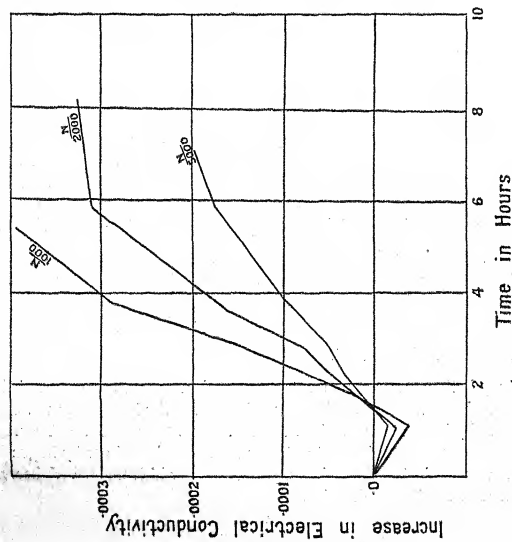
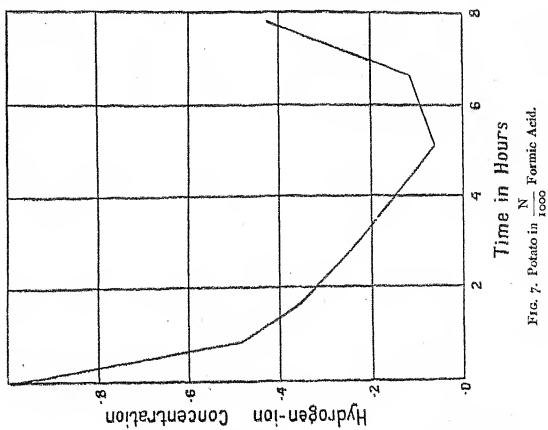


FIG. 6. Potato in Formic Acid.

FIG. 7. Potato in $\frac{N}{1000}$ Formic Acid.

Acetic Acid.

Series 9 and 10.—The next acid of the series, acetic acid, was used in concentrations $\frac{N}{500}$, $\frac{N}{1000}$, $\frac{N}{2000}$, $\frac{N}{5000}$. It will be observed from Fig. 8 that the conductivity of the external solution changes in the same way as in the case of formic acid.

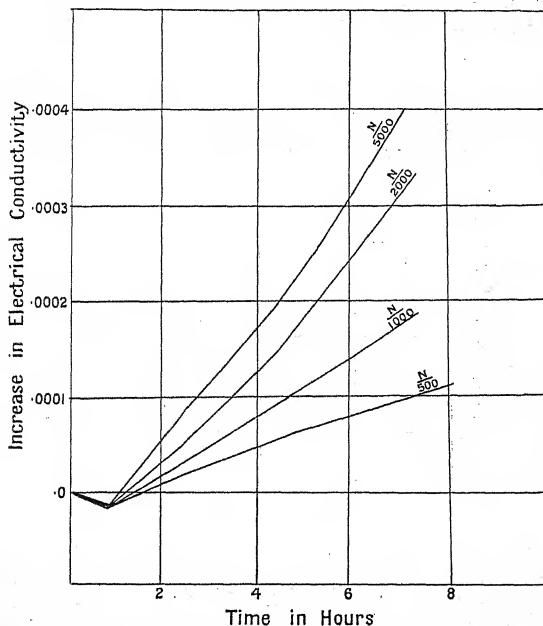


FIG. 8. Potato in Acetic Acid of various strengths.

In Fig. 9 is shown the rate at which the hydrogen ion is absorbed by potato tissue from $\frac{N}{1000}$ acetic acid. The curious rise in concentration of the hydrogen ion, after a few hours of continuous decrease, is shown again here, as in the case of formic acid.

The experiments described above show that considerable difference exists between acids, even in dilute solution, as regards their action on plant cells. The mineral acids on the one hand cause much less exosmosis, and presumably therefore produce much less injury than the two fatty acids employed on the other. Oxalic acid appears to occupy an intermediate position.

The earlier work referred to in the introduction to this paper is not therefore absolutely confirmed. Support is given to the contention that with organic acids the anion influences the action on plant cells, but, having

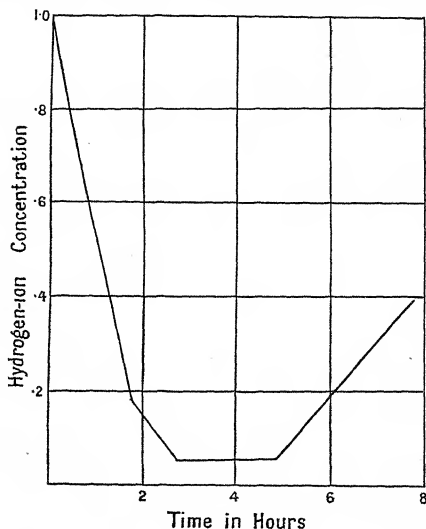


FIG. 9. Potato in $\frac{N}{1000}$ Acetic Acid.

regard to the different results with hydrochloric and nitric acids, it must be supposed that even with these the anion also has an effect. However, the acids containing 'nutrient' ions such as SO_4 and NO_3 , as well as hydrochloric acid, are sharply marked off from the fatty acids in respect of their power of producing exosmosis from the tissues.

A further conclusion which may be drawn from the curves given in this section, is that the rate of absorption of an acid depends on its

concentration, as has already been shown in the second paper of this series (13).

The experiments recorded in which the electrometric method of measurement was used show that an actual absorption of hydrogen ions does take place with all the acids examined. With the mineral acids on the one hand, and the fatty acids on the other, there is, however, a very noteworthy difference. In the case of the former there was a continuous absorption of acid as far as the experiments were carried; with the latter this continued reduction of the acidity of the external solution gave place after a time to a marked increase in the acidity. A discussion of this result follows later in this paper.

II. Experiments with Living Plants of *Vicia Faba*.

In order to determine whether the roots of living plants absorb acids in a similar way to potato cells, some experiments were made with Bean plants (*Vicia Faba*). Seeds were germinated in sawdust, and the young seedlings transferred to water-culture solutions, so that the roots of the experimental plants should be uninjured. Before an experiment the roots of the plants were well washed with distilled water, then the plants were placed with their roots in bottles containing 100 c.c. of acid.

Series 11. In this series, Bean plants were used with $\frac{N}{1000}$ nitric acid.

Both the conductivity and the hydrogen-ion concentrations of solutions were measured after various intervals. Fig. 10 shows the change in conductivity, and Fig. 11 the rate of absorption of the hydrogen ion.

These results indicate that the absorption of acid by living Bean plants takes place in exactly the same way as the absorption by discs of potato tuber.

III. On the Part played by proteins in the Absorption of Acids by Potato Cells.

In order to obtain some information as to which of the various substances present in the cells of the potato are responsible for this rapid absorption of hydrogen ions, potatoes were ground up with sand and subjected to pressure. The expressed liquid so obtained was filtered off. The filtrate contained sugar and proteins, but no starch. The hydrogen-ion concentration of the liquid was measured, and was found to be only slight.

There was now added to 100 c.c. of an $\frac{N}{1000}$ acid solution 1 c.c. of the filtrate, and the hydrogen-ion concentration of the resulting liquid measured. The hydrogen-ion concentration had decreased considerably. An attempt



was made at a partial separation of the colloidal and crystalloidal substances in the expressed liquid, by filtering under pressure in Bechold's ultra-filtration apparatus through a 3 per cent. collodion filter. The filtrate so obtained would contain the crystalloids, but a large part of the colloids would be left behind on the filter. When 1 c.c. of the filtered solution was now added to 100 c.c. acid, there was produced a much smaller decrease in the hydrogen-ion concentration of the acid; thus it appears that the decrease in hydrogen-ion concentration, produced by the crude expressed liquid, is probably due to the action of the colloidal substances in the

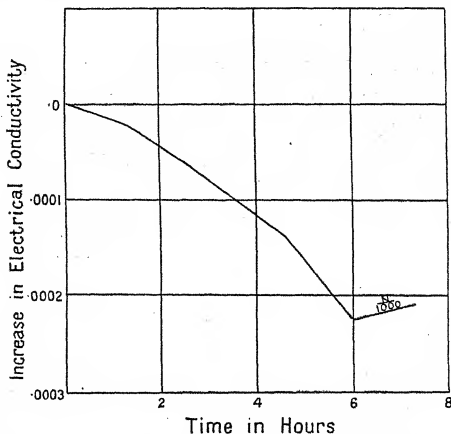


FIG. 10. Bean in $\frac{N}{1000}$ Nitric Acid.

potato extract. Formic, acetic, and nitric acids were used in these experiments. The proteins in some of the solution obtained from the potato were precipitated by means of colloidal ferric hydroxide, and the solution then filtered. 1 c.c. of the filtrate added to 100 c.c. acid produced no effect on the hydrogen-ion concentration of the acid.

As the removal of protein from the solution also removes its power of reducing the hydrogen-ion concentration of acid solutions, it suggests that the absorbing substances of the living cells may be proteins. It is known that proteins react chemically with acids, and will therefore affect their hydrogen-ion concentration.

A solution of peptone in water was therefore prepared, and 1 c.c. of it

added to 100 c.c. of $\frac{N}{1000}$ acid. There was immediately produced a marked decrease in the hydrogen-ion concentration of the acid, very similar to that produced by 1 c.c. of potato extract.

To obtain further proof that in the case of the liquid obtained from the potato it is the protein that affects the acid, the proteins of the potato were extracted. These were found to act in the same way, in regard to

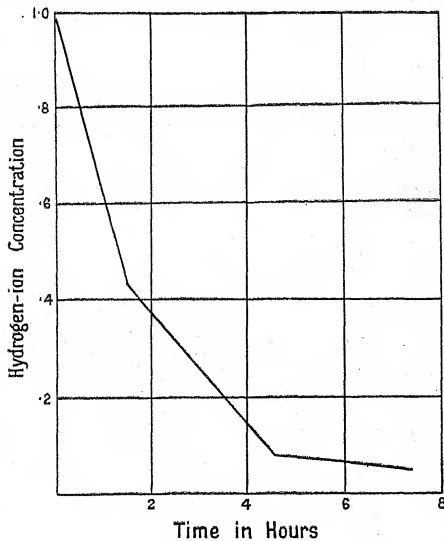


FIG. 11. Bean in $\frac{N}{1000}$ Nitric Acid.

the acid, as did the peptone solution and also the liquid expressed from potatoes.

It has been thought by some investigators that lipoid substances play an important part in the absorption of substances by the cell, and lecithin has been suggested as particularly active in this regard. A colloidal solution of commercial lecithin in water was prepared, and 1 c.c. of this was added to 100 c.c. of $\frac{N}{1000}$ acid; measurements before and after the addition

of the lecithin showed that it had little effect on the hydrogen-ion concentration of the acid.

DISCUSSION.

The results of the experiments described in this paper lead one to conclude that acids in dilute solution, or at any rate their hydrogen ions, readily enter plant cells.

The curves obtained in the electrical conductivity experiments show that plant cells are affected in different ways by different acids, and also give an idea of the rate and the manner in which the acids affect the cells; but considering the complexity of the system, and the number of actions possible, it cannot be assumed that they give a definite measure of either the rate of absorption of the acid, or of the exosmosis of the substances in solution within the cell. They do suggest, however, that in dilute solution some acids can penetrate into the cells without doing much injury to them for some time, while others produce such an effect that some of the substances within the cell pass out almost as soon as the potato discs are immersed in the acid.

The hydrogen-ion concentration measurements show the actual rate of absorption of the hydrogen ions by the cells. From the curves, it can be seen that the hydrogen ions of various acids are absorbed in the same way for some time, but that, after some hours, acids such as formic acid produce results different from those produced by the mineral acids. The anions have obviously an effect, and determine whether the acid is toxic or comparatively non-toxic, toxicity being roughly indicated by the rate of exosmosis of electrolytes from the cell, as indicated by a comparison of the conductivity curves and those of hydrogen-ion concentration.

The increase in the hydrogen-ion concentration of the external solution which takes place after some hours in the case of formic and acetic acid is a curious fact which requires explanation.

It may be supposed that in penetrating into the cell, these acids, which are both chemically active, may react with some of the cell contents and produce other acids, probably organic acids which cannot normally pass through the limiting layer of the protoplast. But in entering the cell, the acid will have probably reacted with the outer layers of the protoplasm, and will have so altered their chemical constitution that the acids produced in the cell are able to diffuse out, and so increase the hydrogen-ion concentration of the external solution.

Considerable interest attaches to the mechanism by which acids enter the cell so rapidly. So far, opinion has been divided as to whether it is the protein or lipid substances which are active in absorption. The experiments described in the third section of the experimental part of this paper all appear to suggest that, in regard to absorption of acids, it is the protein

substances of the cell which are responsible. The only lipid substance examined, crude lecithin, gave no result similar to that obtained with living cells, whereas the resemblance between the action of living cells on acids and that of both peptone and the proteins extracted from potato is striking.

Perhaps too much stress should not be laid on this isolated experiment, for commercial lecithin is a very impure product, and may contain as much as 50 per cent. impurities (8). Nevertheless, even in such a case one would expect a reduction in the hydrogen-ion concentration of the acid, if lecithin absorbed acid in the same way as the cell.

Finally, it should be mentioned that the decrease in the hydrogen-ion concentration of the solutions containing plant tissue cannot be explained by the diffusion out from the cell of protein substances which then react with the acid in the external solution. Tests made for proteins in the external solution failed to reveal their presence there, even after forty-eight hours' immersion in dilute acid. Moreover, it would be extremely unlikely that complex compounds like proteins would diffuse out during the first few hours of immersion in dilute acid.

SUMMARY.

1. The hydrogen ions of all acids examined are rapidly absorbed by plant tissue from dilute solutions.
2. The anion of the acid plays a large part in determining the effect of the acid on the cell, the fatty acids standing in strong contrast to the mineral acids. In the case of the mineral acids the exosmosis of electrolytes produced is considerably less than in the case of formic and acetic acids.
3. Some evidence is brought forward which suggests that proteins may play an essential part in the absorption of acids by plants. No evidence has been obtained suggesting that lecithin is at all active in this regard.

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The Morphology and Anatomy of the Genus *Statice* as represented at Blakeney Point.¹

Part I. Statice binervosa, G. E. Smith, and
S. bellidifolia, D.C. (= *S. reticulata*).²

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With Plate VI, twenty-eight Text Figures, and four Tables.

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IN the summer of 1912, at the suggestion of Professor F. W. Oliver, an investigation of the various species of *Statice* which occur in such variety and profusion at Blakeney Point, Norfolk, was begun; the area shares with the neighbouring Burnham-Brancaster system the distinction of possessing every British species of the genus with the exception of *S. Dodartii* (Gri.).

The present paper is concerned with the forms which are more specially related to the shingle banks and lows, namely *Statice binervosa*, G. E. Smith, and *S. bellidifolia*, D.C., while the species more particularly characteristic of the salt marsh will be dealt with in a later communication.

The floral morphology and ecology of the genus have been investigated by Dr. E. J. Salisbury, to whom the author is entirely indebted

¹ Blakeney Point Publication, Number 15.

² The nomenclature adopted is that used by Mr. C. E. Salmon, Journ. Bot., vol. li, p. 92 et seq. [Annals of Botany, Vol. XXX. No. CXVIII. April, 1916.]

for this part of the paper. It is a great pleasure to acknowledge also the help given me by him in the identifications, in supplying plants raised from seed, and for his assistance in the preparation of the paper.¹

I. HABITATS OF THE PLANTS EXAMINED AND CONSIDERATION OF THE FACTORS INVOLVED.

Since the topography of Blakeney Point has been fully illustrated and described, it will not be further considered here.²

Statice binervosa.

This species usually occupies a very definite habitat. It is most commonly found forming a zone upon the flanks of the lateral shingle banks (Oliver and Salisbury, loc. cit., p. 30 of reprint). In this situation it is the most characteristic species, and occurs in company with *Frankenia laevis*, *Armeria maritima*, *Plantago Coronopus*, and *Glyceria maritima*. These lateral shingle banks occur more or less at right angles to the shore, they are sheltered from wave impact, and are in a state of dormancy; their shingle is stabilized. The soil of this zone is typically bare shingle in which the interspaces are completely filled with sandy mud. The habitat of *S. binervosa* is therefore only reached by the very highest tides, so that this species is the least maritime of any of the *Statices* found upon the area. It is in harmony with this fact that *Statice binervosa* will flourish and flower for years in normal garden soil. The rare occurrence of this species on the Main bank, in which habitat the mobile pebbles of the beach are gradually encroaching on the marsh, indicates that conditions of stability are a necessity for its existence. Oliver and Salisbury record, in this connexion, that *S. binervosa* only occurs on the Main bank sparsely near the crest, where the bank is broad and therefore less mobile, or else where dunes on the seaward face make stability more comparable with that on the laterals; further, on the 'Yankee bank' of the Long Hills the *binervosa* zone is discontinuous, and the breaks correspond with the places where the shingle is unprotected and therefore unstable. In obvious relation to this first requisite of the plant for a fairly stable habitat there is a remarkable difference between its underground parts and those of such typical mobile shingle Main bank plants as *Suaeda fruticosa*, *Silene maritima*, and *Arenaria peploides*. In these latter, rejuvenation by vigorous budding from the prostrated shoots or from the rhizome occurs abundantly, and there is ample evidence to prove that the more the shingling the greater the response of the plant to

¹ Text-figs. 2, 3, 4 and 13, and Photographs 1 and 2 were kindly given by Dr. Salisbury.

² Oliver, F. W., and Salisbury, E. J.: Topography and Vegetation of Blakeney Point, Norfolk. Trans. Norfolk and Norwich Nat. Soc., vol. ix, 1913, p. 485; also reprinted with separate paging.

it.¹ The underground parts of *S. binervosa* consist of a short stout stem, which branches profusely at the apex, and which below passes over into a long, stout root of wire-like consistency. The plant has a rosette habit, and shows no signs of any rejuvenation by budding.

A slightly different habitat, in which *S. binervosa* often occurs abundantly, is the shingle low, a depression left between closely juxtaposed banks; it occurs especially at the convergence of laterals near their junction with the Main bank, and being accessible to the highest tides and at the same time secluded and tranquil, a covering of mud of greater or less thickness becomes deposited over the shingle. The lower part of such shingle lows is the main habitat of *S. bellidifolia*, while on the higher margins *S. binervosa* occurs. The lows are frequently very muddy, but in some parts, where the tide rarely floods the low, and where, owing to the near presence of dunes, abundance of sand is available, the lows may be distinctly sandy. Finally, if the tide is cut off entirely, the *binervosa* zone may gradually spread centripetally amongst the *Suaeda* hummocks. Accumulation of sand, however, soon results in the extermination of the *Statice* and its replacement by the dune flora.

Speaking generally, though the flanks of the lateral banks form the main habitat of the plant, yet the most luxuriant specimens are those from the crest. Here, since the fixity of the shingle is increased, the rate of accretion of the soil is accelerated, and the growth of *S. binervosa* is favoured, until, with the establishment of other plants, the factor of competition comes into play and the *binervosa* zone is driven lower down the flanks, and only relict plants are able to survive on the crest. In this connexion Salisbury gives some interesting data as to the average height and general vigour both of *S. binervosa* and also of the somewhat parallel case of *Plantago Coronopus*. For example, the average height of crest plants of *S. binervosa* is 8.5" as compared with 4.18" for plants from the flanks. The explanation given by Salisbury is as follows: ² 'under the rigorous conditions of the sloping flanks, the *Statice* abounds through the absence of its less hardy competitors, but with the accretion of soil the limiting factor for these latter is removed and only the more robust of the *Statice*s survive the ensuing struggle: these from their perennial character may remain for a considerable number of years, and by virtue of the 2" of soil in which they grow, they will be better nourished and therefore larger than those of the flanks which are rooted in bare or nearly bare shingle.'

Relict plants of *S. binervosa* also occur near the base of a lateral where they become buried by the advancing shingle fans of the Main bank. Such

¹ Oliver, F. W., and Salisbury, E. J.: *Vegetation and Mobile Ground as illustrated by Suaeda fruticosa on Shingle.* Journ. of Ecol., vol. i, 1913, p. 249.

² Loc. cit., reprint, p. 38.

plants afford a striking instance of the luxuriance due to the temporarily improved conditions.

Salisbury suggests that the remarkable increase in size of these overwhelmed plants is probably due to one of two causes, or to a combination of both. He attributes it to the freedom from competition which is procured by the onflow of the pebbles, since the shingle kills most of the plants it covers, or else to the mulch action of the shingle on the buried soil, tending to increase its water-retaining power.

The shingle, as such, is obviously not of value, for the lateral roots of the plants when dug up are found to be practically restricted to the soil beneath the covering layer, though they may also occur in the top layers where the soil and humus collect.



TEXT-FIG. 1. Plant of *Statice binervosa* after shingling. $\times 3$. A, old dead rosette of leaves; B, new rosette.

In order to determine more exactly the effect of shingling on *S. binervosa*, four experimental areas were started on the crest of a lateral shingle bank on the Marams in November, 1912. Patches of the bank about one yard in diameter were covered with loose shingle from the Main bank to a depth of 5 cm. In August, 1913, the areas were examined and in every case the characteristic plants had grown through the shingle covering, viz. *Statice binervosa*, *Armeria maritima*, *Plantago Coronopus*, *Obione portulacoides*, and, in two of the patches only, *Frankenia laevis*. The plants of the covered patches showed a remarkable increase in luxuriance as compared with those of the surrounding bank; not only were the rosettes more spreading, but the size of the individual leaves was much greater, the inflorescence axis much larger, and the flowering heads were distinctly more luxuriant. These features occurred not only in the case of *Statice*, but also in plants of *Armeria* and *Plantago*, and to a much less marked extent in *Obione* and *Frankenia*. Further, while practically all the plants of *S. binervosa* on the bank showed distinctly red coloration of the leaves, those of the covered patches were vividly green, with no trace of red.

On removing plants of *Statice* and *Armeria* for examination, it was seen that all the leaves of the old rosette had died, and had been replaced by a vigorous output of new leaves from one of the shoots of the rosette.

After examination of the areas, three of the experimental patches

(1, 3 and 4) were provided with a further 5 cm. coating of loose shingle, while the fourth area (2) was left untouched.

In August, 1914, the patches were again examined, with the following results:

Area 2.—Coated with 5 cm. of shingle in 1912 and untouched afterwards.

Only one isolated *Statice* had survived; it was distinctly more luxuriant than the plants on the untouched bank around, and its leaves were not only longer but greener. The inflorescence was distinctly more branched and luxuriant, and two spikes of flowers were present instead of the one usual in the uncovered plants. The length of the inflorescences was 12 cm. and 13 cm. respectively, while the average length of the inflorescence in the uncovered plants was only 5 to 7 cm.

Areas 1, 3 and 4.—Covered with 5 cm. shingle in 1912 and a further 5 cm. in 1913.

The additional 5 cm. of shingle had in every case proved fatal to all the plants of the area, except a single runner of *Agropyron* sp., which had survived the additional shingling.

The margins of the experimental areas had escaped the second shingling, and here the plants were covered with only the original 5 cm. of pebbles added in 1912. In this region the plants were numerous, and as regards their inflorescences much more luxuriant. The *Obione*, *Plantago*, and *Armeria* were, on the whole, distinctly more flourishing than the plants of the uncovered bank, and *Obione* seedlings were numerous. The *Statice* plants had very few leaves, and though the inflorescences were more vigorous, the plants had the appearance of making a final effort before death ensued.

The average length of the inflorescence axis of *Statice* in the uncovered area surrounding the patch was 6 cm., in the covered patch the average height was 18 cm., while in one case the length attained was 35 cm.

An examination of the ground, on a hot afternoon after a day of brilliant sunshine, revealed the fact that in the experimental areas, though the surface shingle was dry, 2.5 cm. below the surface the stones were quite damp, an observation which is in agreement with the results of Oliver and of Hill and Hanley.¹ Beneath the coating of shingle the ground was quite dry, and on the adjacent untreated lateral both the surface and 2.5 cm. below were absolutely dry.

In the experimental areas the effect of the loose shingle was similar upon all the species covered except *Agropyron*. It appears as though the

¹ Oliver, F. W.: The Shingle Beach as a Plant Habitat. New Phytologist, vol. xi, 1914, p. 98.
Hill, T. G., and Hanley, J. A.: The Structure and Water-Content of Shingle Beaches. (Blakeney Point Publ., No. 11), Jour. Ecol., vol. ii, 1914, p. 35.

increased luxuriance is due in these cases largely to the mulch action of the advancing fan, which acts by retaining a damp zone around the growing points of the plants, enabling them to grow for a time with greater vigour. Eventually, it would appear that the rapid raising of the surface level produced by still further additions of shingle proves disastrous for a plant whose only method of combating the attack is to increase the length of the petiole in the effort to bring the leaves well above the surface again.

It would thus appear that the restriction of *S. binervosa* to its very limited habitat depends entirely on the ill-equipment of its underground parts. It is unable to colonize mobile shingle, not because of the arid conditions and lack of humus, but because of its inability to rejuvenate when covered, and because, as will be seen later, the structure of the stem is unsuited to vigorous branching accompanied by rapid growth, and on the other hand it cannot compete with other colonizers of the crest of the stable laterals, since the centrifugal extension of the turf of the various grasses, or of rhizomatous plants, or of cushion plants subjugates it, hence it is practically restricted to the narrow belt of 'No Man's Land' on the sloping flanks of the laterals, where the conditions are probably more rigorous in many respects than in any other part of the area, and where it reigns supreme. Here the water-supply is extremely limited on account of the slope, the food-supply is small, for the accumulation of humus is comparatively slight, and exceptionally high tides may deposit a coating of mud over the plants; as will be shown later, the anatomy of the plant is well fitted to enable it to endure all these adverse conditions.

Statice bellidifolia (= *reticulata*).

This species invariably occurs in situations reached by all but the lower tides, and especially in the muddy shingle lows already described, which remain moist throughout the inter-tidal period.

The conditions of life in these lows is thus very different from those on the sloping flanks of a lateral, and approximate at certain times to those of a salt marsh, where, too, the plant is sometimes to be found. After a very high tide the salt water stands in the lows, converting them into small lagoons from which the water escapes only very slowly by percolation;¹ the tide is left in them long after the *Pelvetia* and *Aster* marshes become fully exposed again. If the tides are exceptionally high and evaporation low, they may not become empty between two successive tides. It is clear that after a period of such unusually high tide, the salt water will remain standing in the lows for some time, so that *S. bellidifolia* will have to face concentrations of salt water, and will thus be exposed at more or less regular intervals

¹ My thanks are due to Dr. Sarah Baker, who kindly made these observations for me during a visit to Blakeney in November, 1913, in order to study the especially high November tides of that year.

to prolonged periods of salt marsh conditions, while at other times they will approximate more nearly to those of the flanks of the laterals. It is in accordance with this unusual combination of factors that one meets in the anatomy a mixture of characters in many respects recalling, on the one hand, those of the closely related *S. binervosa*, and on the other, those of the typical salt marsh forms such as *S. Limonium*.

It is of interest to note that Long¹ gives the habitat of *S. bellidifolia* in the adjacent marshes at Wells as rolling sand-covered knolls 2 to 3 ft. above the highest spring tides. The much larger size of these specimens here and at Burnham Overy is probably due to the great depth of the sandy mud in which they grow as compared with the shallow mud overlying the shingle in the Blakeney 'lows'.

In the region of the Long Hills and on the shingle plateau on the north-west side of the upper reaches of the *Pelvetia* marsh, where it approaches the main shingle bank, the localities of *binervosa* and *bellidifolia* overlap to a certain extent, and in these regions a form of *S. binervosa* occurs which differs in certain respects from the typical one. In many features, both morphological and anatomical, this form, distinguished as the 'broad-leaved *binervosa*' in contradistinction to the typical narrow-leaved plant, is intermediate between *S. binervosa* and *S. bellidifolia*.² There is a possibility that we are dealing with a hybrid between the two, though attempts to raise the plant from seed have so far failed. The broad-leaved type is entirely absent from the region of the Marams, where also only *S. binervosa* occurs, and is restricted to those regions where the possible parent species are both present.

In addition to the plants whose habitats have been briefly described above, plants from experimental garden cultures started by Dr. E. J. Salisbury have also been examined, more particularly *S. binervosa* plants grown in a cold greenhouse, and plants raised from seed.

A summary of the chief forms of *S. binervosa* examined is as follows:

1. *Tall Form*—luxuriant plants growing on the edge of a shingle fan, termed 'Main bank plants'.
2. *Dwarf Form*—(Pl. VI, Photo 1)—
 - (a) Typical *S. binervosa* plants from the lateral shingle banks, termed 'narrow-leaved *binervosa*'.
 - (b) Plants from the margin of the muddy shingle lows, termed 'mud plants'.
 - (c) Plants from sandy shingle lows, termed 'sand plants'.
 - (d) Plants from the experimental areas on the lateral shingle banks.

¹ Long, F.: The Salt-Marsh Flora of Wells. Trans. Norf. and Norw. Nat. Soc., vol. viii, p. 523.

² Blakeney Point in 1914, p. 15; also Trans. Norf. and Norw. Nat. Soc., vol. x, p. 65.

(e) Plants cultivated in a greenhouse or raised from seed, termed 'culture plants'.

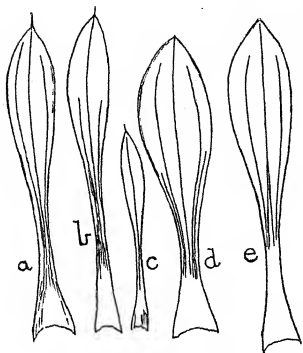
3. Plants of a type which only occur where *S. binervosa* and *S. bellidifolia* meet, termed 'broad-leaved variety' (Pl. VI, Photo 2).

II. DESCRIPTION OF THE SPECIES.

A. *Statice binervosa*.

The great variability of this species has already been the subject of comment. As Mr. C. E. Salmon has pointed out: 'Almost every locality for *L. occidentale* (= *S. binervosa*) in Britain seems to possess

a form slightly different from the 'plants in another locality.'¹ At Blakeney several forms are to be found, but these fall into two groups, viz. the typical narrow-leaved form and the broad-leaved variety which may possibly be of hybrid origin.



TEXT-FIG. 2. Leaves of *Statice binervosa*.
a-c, narrow-leaved form; d-e, broad-leaved form.
Nat. size.

Within certain definite limits the narrow-leaved form shows variation. The width of the leaves, for example, appears to be correlated with the amount of moisture available. Thus if plants be grown in very dry soil the lamina tends to become very narrow (Text-fig. 2, c), whereas the width increases if abundant water is supplied (Text-fig. 2, a). In the latter case, however, the majority of the leaves have excurrent veins,

thus differing from the broad-leaved variety. Cultivation and supply of moisture seem not to affect the floral characters to any appreciable extent. If conditions are moist, however, the purple coloration of the bracts appears to be absent. The difference in height seems to be mainly a question of nutrition and not dependent on heredity.

The Leaves.

In the adult condition the leaves of *Statice binervosa* (Text-fig. 2) are lanceolate-spathulate, the blade narrowing to a long petiole. The width and form of the blade vary greatly and the two main types will be considered

¹ Salmon, C. E.: Journ. of Bot., vol. xli, 1903, p. 70.

later in relation to the forms with which they are associated. Normally the tip is acute and terminates in the excurrent midrib, which is a bristle-like process sometimes nearly 2 mm. in length. In other leaves upon the same plant, however, this may be so short as to be practically absent. The leaf is bordered by a narrow hyaline margin that, towards the base of the petiole, broadens out on both sides to form the sheathing leaf base. The latter bears mucilage-secreting glands.

The venation usually consists of a prominent midrib accompanied by two lateral veins that in the petiole run parallel to it. In the blade the lateral veins diverge somewhat and usually disappear at about the region of maximum width. In the upper part of the blade two or more small veins are sometimes visible arising in a pinnate manner from the midrib. In the narrower types of leaf the lateral veins may be altogether absent.

The leaves, from the absence of internodes, constitute a rosette which is pressed close to the ground. At first the leaves are erect and in this position their sheathing bases protect the younger foliage. In the juvenile state the lamina is rolled inwards parallel to the midrib. As the petioles elongate the blades spread outwards, unless the plant be protected as when surrounded by other vegetation, and a closely adpressed rosette results, in which condition the winter is passed. The leaves of any one season usually persist until late into the season following.

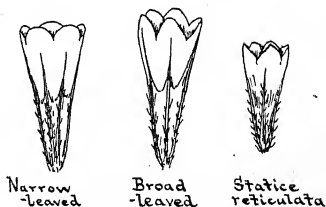
The Scape.

The scapes are usually erect with ascending branches; the latter all arise at an acute angle with the axis from which they originate. The scape is usually branched from below the middle, and the primary branches of the inflorescence mostly lie in one plane.

Sterile branches are few or entirely absent; the spikelets are crowded and form two imbricated rows. The interval between successive spikelets on the same side of the axis is usually less than two-thirds the length of the spikelet. Each spikelet contains from one to three flowers, of which one, however, may be abortive.

Typically the scape is only scabrid-pulverulent, due to conically projecting cells, in the *lateral branchings* or even smooth throughout.

Flowering period. From the middle of July to the end of August.



TEXT-FIG. 3. Calyces of *Statice binervosa* and *S. reticulata* (= *bellidifolia*). Enlarged.

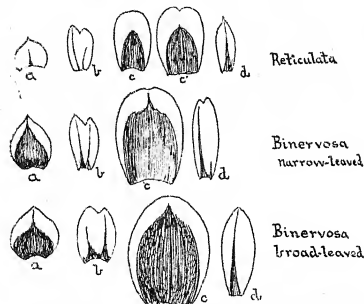
Flowers.

The flowers are deep reddish purple or pale violet in colour with a decidedly redder tinge than those of the other species. When fully open they measure from 5.5 to 7 mm. in diameter.

Petals clearly notched, about 7 mm. long. Each with a broad reddish vein extending nearly to the tip.

Anthers pale yellow.

Calyx with short and blunt, almost truncate, teeth (Text-fig. 3) without intermediate teeth, the veins ending in reddish tips. Usually with three hairy ribs and two glabrous ribs with closely adpressed hairs usually present between the hairy ribs.



TEXT-FIG. 4. Bracts and bracteoles of *Statice binervosa* and *S. bellidifolia*. Top row, *S. reticulata* (= *bellidifolia*); middle row, *S. binervosa*, narrow-leaved form; bottom row, *S. binervosa*, broad-leaved variety. a, outer bract; b, middle bract; c and c', inner bract; d, bracteole. $\times 4$.

bract, membranous, asymmetrically bilobed or nearly entire, oblong, broadest just below tip, with usually two unequal veins (Text-fig. 4, b).

Inner bract. Oval to broadly oval, blunt, opaque, green or slightly purplish with a broad membranous margin. About twice the length of the outer bract. Usually about 5 mm. long by 4 mm. in broadest part (Text-fig. 4, c). Sometimes notched (Text-fig. 4, c, middle row).

Bracteole. Oblong asymmetrical with a rounded or slightly notched tip and a single vein to one side (Text-fig. 4, d).

Seed. Brown, lanceolate, smooth, about 2 mm. long.

Scale leaves. Triangular acuminate.

Outer bract (Text-fig. 4, a). Broad ovate tapering from just above the base to an acute apex, green or tinged with purple especially in the drier situations. Central part opaque, passing into a narrow acutely ending vein at tip. The margin broad hyaline and usually colourless or very faint. From 2 to 3 mm. long and slightly less in width.

Middle bract. A trifle shorter than the outer

The Broad-leaved Variety (Pl. VI, Photo 2).

This form is almost invariably found growing in the neighbourhood of the normal drift line. Its habitat is, so far as level is concerned, thus intermediate between that of *Statice bellidifolia* on the one hand and *S. binervosa* proper on the other. In fact, it has as yet only been found at Blakeney, where these two species meet. The most noticeable differences from *S. binervosa* are the form of the leaves and inflorescence.

The leaves are broader than the type (Text-fig. 2, *d, e*) and are usually without the excurrent vein. The lateral veins are generally well developed, and sometimes four are present in place of the normal two.

The scapes are more or less spreading and branched from near the base, but the branches on the lower half are usually sterile. The branches of the scape are usually scabrid-pulverulent down to the base. Branches much more divergent than in the type and approximating less to one plane. Spikelets crowded, imbricate, with from 1 to 4 flowers. Calyx teeth longer and more rounded than those of the type, with from 2 to 4 hairy ribs.

The bracts and bractcoles larger. Inner bract usually from 5.5 to 6 mm. long and about 4.5 mm. broad.

The flowering period is earlier than that of the type form, the plant being usually over bloom by the middle of August.

B. *Statice bellidifolia*.

The leaves. The leaves are petioled, the slender petiole often exceeding the blade in length. The latter is narrowly obovate-lanceolate. The apex is frequently blunt or ends in a very short mucronate tip. The leaves generally wither away before flowering, previous to which they often take on a deep reddish brown or purple colour. The margin is hyaline but very narrow. Usually but a single vein is present, though two lateral veins may be faintly developed.

Owing to the early withering of the leaves they afford little or no protection to those of the following season. In actual fact the new season's foliage can already be found during the winter as a number of 'winter buds', consisting of closely packed leaves, about 6 to 10 mm. in length. As a result of the plant's habitat these buds are usually adequately protected by the silting up of sand and mud around the crown of the plant. The size of the entire plant varies enormously. Thus the crown in young specimens may be unbranched and not more than $1\frac{1}{2}$ cm. in diameter. Such specimens usually only produce sterile scapes. In larger specimens the crown alone may be 7 to 8 cm. in diameter.

The largest specimens found at Blakeney had a spread of (including the scapes) about 40 cm., but specimens from the marshes at Burnham Overly

measured over 48 cm. in diameter. One of these latter bore no less than forty-two independent scapes, at the base of each of which there were from 3 to 5 winter buds developed in the axils of withered leaves. Thus, potentially, there were well over 120 shoots of the season following.

As in *S. binervosa* the inflorescences are terminal in position.

The scape. The scapes are scabrid-pulverulent throughout. They are richly branched from near the base, but only the apical branches are fertile. The main axis of the scape is usually prostrate, the lateral branches ascending divergent and *not approximating* to one plane.

The scale leaves are acute, membranous, brown, or lower foliaceous.

The spikelets are more or less imbricated, with from 1 to 2 flowers. The interval between successive spikelets on the same side usually $\frac{1}{4}$ to $\frac{1}{2}$ the length of the spikelet.

Flowering period. July.

Flowers. Pale pinkish purple, from 3 to 4 mm. in diameter. Petals usually entire, blunt or very slightly emarginate. Midrib coloured as blade or a very little darker.

Anthers yellow.

Calyx (Text-fig. 3). Usually about 3 to 4 mm. long, with no intermediate teeth. Calyx segments acute with deep purple ribs. All the ribs usually hairy. Long hairs present between the ribs, especially at the base of the intercostal sinuses.

Outer bract. Membranous ovate, obtuse or abruptly acute, with a prominent brown vein from 1.2 to 2 mm. long. Usually about 1.5 mm. (Text-fig. 4, *a*, top row).

Middle bract. Membranous, asymmetrical, obovate, usually bifid, with two unequal veins. Usually 2 to 3 mm. long (Text-fig. 4, *b*).

Inner bract. Obtuse obovate or faintly bilobed, with a broad membranous margin above the middle. The brown opaque portion seldom extending to the apex as a dark line from 3 to 3.8 mm. long (Text-fig. 4, *c*).

Bracteole. Membranous, oblong, asymmetrical, 2 to 3 mm. long (Text-fig. 4, *d*).

Seed. Lanceolate, smooth, brown, about 1.5 mm. long.

III. ANATOMY.

The order Plumbaginacae has been the subject of much anatomical investigation, which has, however, been directed more particularly towards the anomalous structural features of the axis found in the genera *Acantholimon* and *Aegialitis*, and in the investigation of the epidermal glands which occur throughout the order. So far as it has been possible to ascertain, no examination of the anatomy of the *Statice binervosa* has been previously undertaken, and, with the exception of a brief description

of the leaf and scape by Chermezon,¹ *S. bellidifolia* also has apparently not been investigated.

The Glands.

The epidermal glands, which are so characteristic a feature of the order, appear to be identical in structure throughout the British species of *Statice*, and will therefore be considered together for the various forms of *binervosa* and for *S. bellidifolia*.

The glands are of two distinct types, which apparently differ essentially in their structure, and which are differently distributed; every transitional stage, however, exists between the two forms.

Mucilage Glands.

The first type was termed the mucilage gland by Wilson,² who described it as occurring in all the eight genera of the Plumbaginaceae, though not in all the species investigated by him.

He states that it consists of a secreting head borne on a base consisting of relatively few stout walled cells. The secreting cells are numerous, and extremely thin walled, they are prismatic, columnar or conical in shape, and may or may not be divided by a few transverse septa; their contents are finely granular (Text-fig. 6, A and B). He describes them as occurring in the axils of the leaves, and regards them as functioning in the secretion of mucilage. He considers that the mucilage is for the purpose of protection,³ its hygroscopic power being of service in attracting, storing and economizing atmospheric moisture, though at the same time he regards it difficult to explain its presence in the leaf axils of *Armeria* sp. and *Statice* sp., where the plants are living in marshy situations, with the bases of their leaves in contact with the humid soil.

With regard to the species under examination in the present communication, the distribution of the mucilage glands is similar to that described by Wilson. At the extreme base of the leaf sheath on its upper surface in *S. binervosa*, in all the forms examined, mucilage glands are very abundant, forming in parts an almost continuous layer over the sheath. As the distance from the leaf axil increases, the glands become fewer in number, and finally pass over by every stage of transition into the second type of gland.

¹ Chermezon, H.: Recherches anatomiques sur les plantes littorales. Ann. Sci. Nat., Bot., 1910, sér. 9, t. 12, p. 117.

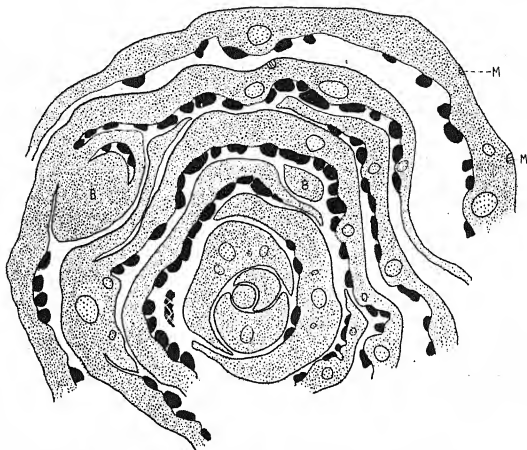
² Wilson, J. T.: The Mucilage- and other Glands of the Plumbaginaceae. Ann. Bot., vol. iv, 1899, p. 231.

³ Details relating to the protection of younger foliage by the leaves are given on pp. 247 and 249.

Some idea of the abundance of these mucilage glands can be gained from Text-fig. 5, which is taken from the apex of a Main bank plant. The effect of this abundantly secreting surface in keeping the apex and buds bathed in mucilage must be of enormous value in preventing desiccation by checking too rapid transpiration.

In *S. bellidifolia* the mucilage glands are few in number as compared with *S. binervosa*.

While in full agreement with the general observations of Wilson as



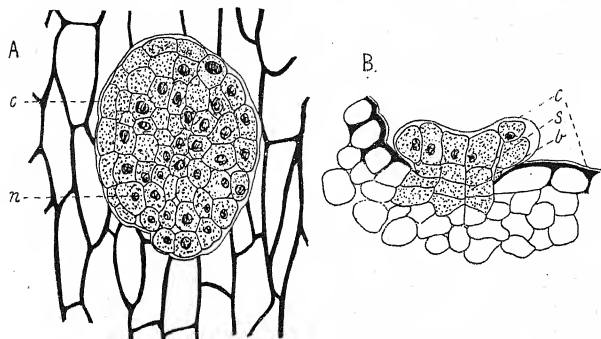
TEXT-FIG. 5. Transverse section of part of the apex of a main bank plant of *S. binervosa*. $\times 36$.
Mucilage glands black; vascular bundles dotted; B = bud; M = Mettenius gland.

to the structure of the mucilage glands, certain details may be added to his description.

Concentrated sulphuric acid has no action on the gland cells, while chlor. zinc. iodide produces a deep brownish-yellow coloration, so that the cells appear to be cuticularized (Text-fig. 6, c). This cuticularization of glandular tissue recalls the similar phenomenon described by Salisbury¹ in the extra-floral nectaries of the genus *Polygonum*, and further the glands figured by him show a close resemblance to the mucilage glands of the Plumbaginaceae. (Compare Figs. 2 and 4 of Salisbury's paper with our Text-figs. 6 B and 6 A.)

¹ Salisbury, E. J.: The Extra-Floral Nectaries of the Genus *Polygonum*. Ann. Bot., vol. xxiii, 1909, p. 237.

As has been noted by Wilson, the gland cells are very rich in protoplasm, finely granular in appearance, and they contain a very large nucleus. The mucilage secreted by the hairs appears to be associated with tannin, a phenomenon which does sometimes occur in the case of mucilage secreting hairs;¹ moreover tannin is commonly abundant throughout the plant. The physiological significance of tannin in the life of the plant is a difficult question, and its function appears to vary in different species.² Sachs concluded that tannin resulted from intense metabolism such as occurs in rapid tissue formation, in vegetative apices and in association with secreting organs, and its presence in the mucilage glands of *Statice* may be accounted for in this



TEXT-FIG. 6. Mucilage gland from the base of the leaf sheath of *S. binervosa*. $\times 290$. A. = gland in surface view; B. = gland in transverse section. c. = cuticle; s. = stalk-cells; b. = basal cells; n. = nucleus.

way. The question of the further distribution of the tannin throughout the plant will be dealt with later.

*Mettenius or Licopoli Glands.*³

The second type of gland occurs on both surfaces of the leaf and on the inflorescence axis of all members of the order.

The structure of the gland was incorrectly described by Mettenius⁴ in

¹ Haas, P., and Hill, T. G.: An Introduction to the Chemistry of Plant Products. Longmans, Green & Co., 1913, p. 125.

The following microchemical reactions invariably gave good results:

(a) A strong aqueous solution of potassium bichromate gave a brownish coloured precipitate.

(b) A neutral solution of ferric chloride gave a blue-black coloration.

² Loc. cit., pp. 214-221.

³ These glands are commonly called chalk glands, since in some species calcium carbonate is excreted by them; this does not occur in any of the British species of *Statice*.

⁴ Mettenius: Filices Hort. Bot. Lips., 1856, p. 10. (Not consulted.)

1856 as consisting of a group of four cells, a mistake in which he was followed by the subsequent workers, Licopoli¹ and Maury.²

The glands were described by de Bary³ as arising from epidermal cells which were rounded quadrate in surface view. Two intersecting walls at right angles to one another, and perpendicular to the surface, divided each of the gland-mother-cells into four; each of the cells thus formed is then divided by a vertical wall into a narrow inner, and a peripheral cell. The eight cells thus produced constitute the gland proper, the walls between the cells are extremely thin, and the contents are composed of very finely granular protoplasm. Volkens⁴ states that the walls which limit the gland towards the inner tissue of the leaf are somewhat thickened, and are distinguished by the fact that they do not swell up or dissolve under the action of concentrated sulphuric acid. He further describes the occurrence of special 'nebenzellen' outside the gland cells; these 'nebenzellen', which are not mentioned in de Bary's description, may be level with the epidermis, or may be deeper and appear as half-moon shaped appendages of the gland elements; he regards them as epidermal cells which were displaced from their original level on the formation of the gland.

Vuillemin⁵ also describes the glands as composed of eight thin walled secreting cells, surrounded by four subsidiary cells whose walls are not dissolved by treatment with boiling potash; the margins of these cells are marked by cutinized attachments which join them to the base of the gland. He further states that 'ces arêtes sont légèrement carénées et pourvues de deux expansions latérales, exactement appliquées sur la commissure qui sépare les cellules annexes', so that the latter form an uninterrupted barrier between the parenchyma on the one hand and the epidermis on the other, nothing passing from one to the other except through these subsidiary cells. He further states only four of the eight glandular cells are secretory, though exchanges can readily take place between all of them on account of their thin walls.

The description of the glands given by Solereder⁶ differs from Vuillemin's account in that the walls of the glandular cells separating the internal surface of the gland from the neighbouring tissues are described as being suberized, and the subsidiary cells are stated to have a double cap instead of a single one.

¹ Licopoli: Gli stomi e le glandole. Atti R. Accad. d. Sc. Fis. e Mat., vol. viii, 1879.

² Maury, P.: Études sur l'organisation et la distribution géographique des Plumbaginacées. Ann. Sci. Nat., Bot., sér. 7, t. 4, 1886, p. 1.

³ de Bary: Vergl. Anat., 1877, p. 113.

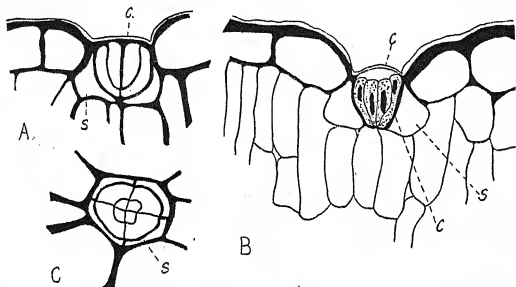
⁴ Volkens, G.: Die Kalkdrüsen der Plumbagineen. Ber. deutsch. bot. Gesell., 1884, Bd. 2, p. 334.

⁵ Vuillemin, P.: Recherches sur quelques glandes épidermiques. Ann. Sci. Nat., Bot., 1887, sér. 7, t. 5, p. 152.

⁶ Solereder, H.: Systematic Anatomy of the Dicotyledons. Oxford, Clarendon Press, 1898, p. 496.

The structure of the glands of the British species of *Statice* agree in most respects with the description given by Vuillemin, for they are invariably composed of a group of eight gland cells surrounded by four subsidiary cells (Text-fig. 7, A, B and C).

In all the forms examined the whole of the walls which separated the subsidiary cells from the gland cells was cutinized, and further the cuticle was continuous across the external surface of the gland; thus the entire gland was encased in a cuticularized layer (this is well shown in Text-fig. 7, B), and treatment with concentrated sulphuric acid left this case intact, but dissolved away the rest of the gland. It follows from this that escape of fluid from the gland will be accomplished with extreme difficulty, a fact which must be of considerable advantage to plants living under conditions where the water available for absorption is very limited in amount, e.g. *S.*



TEXT-FIG. 7. Mettenius gland from the leaf of *S. binervosa*. A. = gland in transverse section; B. = gland in transverse section showing the cuticle raised; C. = gland in surface view. c. = cuticle; s. = subsidiary cells. All \times circa 150.

binervosa, or where absorption is rendered difficult on account of the presence of salt in the water, e.g. *S. Limonium*.

As has already been noted by Wilson, glands showing every stage of transition from mucilage to Mettenian glands are met with in abundance in passing upwards from the leaf sheath to the blade, and Wilson regards both these glands as having the same origin, the Mettenian being the primordial form. He considers that the Mettenian glands were probably mucilage secreting organs, the chalk secretion which occurs in some species of *Statice* having been acquired later. Moreover he states that the contents of the Mettenian glands are always of a mucilaginous nature, even when the gland functions as a chalk-secreting organ. It appears more probable that the mucilage gland, which represents a multicellular trichome consisting of a secreting head borne on a short stalk, is the primi-

tive structure. From such a form, by the embedding of the gland in the leaf tissue and the consequent necessary modification of the stalk-cells, such a type of mucilage gland as occurs in *Aegialitis annulata*¹ is arrived at. By the limitation of the number of secreting cells to eight, and the consequent modification and reduction of the stalk cells to the four subsidiary cells,² the type of Mettenian gland characteristic of *S. binervosa*, &c., is obtained, in which the secreted substance is water and not mucilage. Finally, from such glands, the various modifications found in the chalk glands of such species as *Limoniastrum monoptetalum* can be readily derived.

Two views have been put forward as to the working mechanism of the Mettenian gland. According to Licopoli and Maury the product of secretion is amassed in a space which results from the separation of the four internal glandular cells, and it is rejected by the tension of the cells, which, however, always remain joined by their lower parts. This view appears to be incompatible with the structure of the gland as described by later workers.

de Bary, Volkens, and Woronin all agree that the case is one of simple osmotic phenomena, and Volkens states that the glands act as valves which become efficacious as soon as the transpiration of the aerial organs is in excess of the absorption of water by the roots.

The number of glands present on the leaves of the various forms is shown in Table I. The portion of the leaf chosen was, in each case, the broadest region of the blade, and the numbers in each case represent the average of a number of counts.

TABLE I. Average Number of Mettenian Glands per sq. mm. of Surface.

	Upper surface of leaf.	Lower surface of leaf.
<i>S. bellidifolia</i>	9.6	8.3
<i>S. binervosa</i> , 'broad-leaved' . . .	8.3	9.6
Dwarf Form. <i>S. binervosa</i> :		
mud plant	7.5	12.4
sand plant	9.6	11.7
culture from seed	6.9	9.6
narrow-leaved lateral plant . . .	6.2	8.9
experimental plant	6.2	6.9
binervosa zone of a lateral (one year in greenhouse) . . .	4.8	6.9
crest of a lateral	4.1	4.1
(one year in greenhouse)		
Tall Form. <i>S. binervosa</i> , Main bank plant . . .	5.5	5.5

The glands, speaking generally, are somewhat more numerous on the under than on the upper side of the leaf. It is interesting to note that in the case of the various forms of the dwarf *binervosa*, in which the amount of water available for absorption would be expected to be

¹ Solereder, Fig. 113, D-F, p. 497.

² Or possibly by the elimination of the stalk cells, and the modification of neighbouring cells to form the subsidiary cells.

slightly more than in the typical narrow-leaved plant, the number of glands is slightly greater; compare, for example, the plants from the muddy and sandy lows, and the culture plants, with the type form. Those plants which have been removed from their original home on the lateral shingle bank and have been for a year in cultivation under distinctly more favourable conditions for root absorption show a smaller number of glands per unit area than in the case of the typical form—a condition to be expected, since any increase in the size of the leaf originally laid down would merely tend to spread out the glands, and would not lead to the development of new glandular structures in a leaf already developed. The same explanation possibly accounts for the difference between the gland counts in the case of the tall form from the Main bank and the type dwarf form from the lateral bank.

Finally, it is interesting to note that though the number of glands per unit area in the case of the broad-leaved *S. binervosa* is intermediate, for the upper surface of the leaf, between that of its possible parent forms, no such relation obtains for the lower surface.

STATICE BINERVOsa.

1. *The Seedling.*

The seeds of *S. binervosa* are probably often distributed by very high tides, and seedlings obtained from Blakeney in 1913¹ were found growing in mud among a tangled mass of *Rhizoclonium* filaments. Since seedling plants are not very commonly met with the species may be a 'shy seeder', or else it is only when an exceptionally high tide occurs at the season for seed dispersal that seeds are scattered high enough up on the flanks of the laterals for a safe and stable place of germination to be obtained. This is the more probable in consideration of the facts stated with regard to the spread of *binervosa* near the *Pelvetia* marsh.² The seedlings would appear to grow with extreme slowness, for seedlings collected in August, 1913, showed comparatively little advance on those collected in April of the same year.

The seedlings of *S. binervosa* may be found in considerable numbers amongst the pebbles in the *binervosa* zone during March. Each possesses a pair of narrow, entire, and slightly spatulate cotyledons (Text-fig. 8), with very blunt apices about 4 to 5 mm. in length and from 1 to 1.5 mm. broad in the widest part. In colour the cotyledons are sometimes green, but are more usually of a deep crimson or port-wine colour, which is often shared by the first formed leaves. There is no external indication of a midrib. The cotyledons fuse at the base to form a very short tube.

¹ 1913 seemed to be an exceptionally good year for seedling *binervosa*.

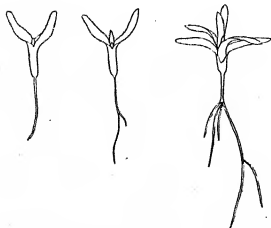
² Report on Blakeney Point for 1914, in Trans. Norfolk and Norwich Nat. Soc., vol. x, p. 65.

The hypocotyl, like the cotyledons, is red in colour and varies in length from 3 to 5 mm. It exhibits a marked contraction at the collet where it passes into the root.

Occasionally tricotylous or even monocotylous seedlings are met with.

The first pair of leaves are shorter and broader than the cotyledons and have very acute apices. It is not usually, however, until the second pair of foliage leaves is produced that distinct excurrent veins are developed at the tips.

The cotyledons have each a very small endarch collateral bundle



TEXT-FIG. 8. Seedling plants of *S. binervosa*.
X 2.

throughout their main portion, but just at the base two very small lateral ones appear. The two main cotyledonary strands enter the axis as endarch collateral structures, the lateral strands also enter and those from the opposing cotyledons fuse, pass towards the centre of the axis, and sooner or later die out. The two cotyledonary strands organize a diarch root according to van Tieghem's Type 3, but no regular 'bifurcation' and 'rotation' of the bundles occur, and the change from stem to root

structure takes place in an ill-defined manner and very rapidly.

Mucilage glands are numerous in the axils of the cotyledons, and Mettenius glands are also present on their surface.

Root hairs occur, but are not numerous.

2. *The Root.*

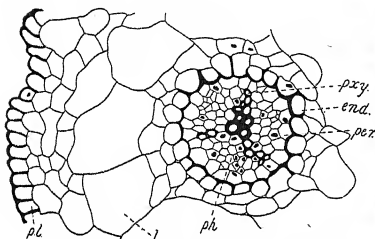
The root system of *S. binervosa* usually varies in size more or less in proportion to that of the aerial organs.

Thus the average height of plants taken from the crest of the laterals and the *binervosa* zone were respectively 22 cm. and 10.5 cm., the average rooting depths being 8.5 cm. and 4.5 cm. Tall plants of the Main bank had a rooting depth of from 18 to 26 cm. There is a main taproot which usually grows obliquely downwards. Large branches are few, but numerous fine laterals are developed which, like the main root, are characterized by their wire-like consistency.

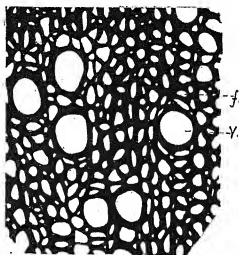
In the stele of the root of the young seedling the xylem occurs in the form of a diarch or triarch plate, with two or three alternating phloem groups. The pericycle consists of a single layer of thin walled cells, while the endodermis is well marked, with thickened inner and radial walls. The cortex is distinguished by its possession of a single ring of

very large thin-walled cells (*l*), and the radial and outer walls of the piliferous layer (*pl*) are thickened and suberized. The structure of a typical seedling root is shown in Text-fig. 9.

At a very early age, while the seedling is still in the cotyledon stage, secondary growth begins, and secondary xylem, consisting of pitted vessels in which, in longitudinal section, the cross-walls are often persistent, and lignified fibres are produced, resulting in the production of a strongly lignified core. No parenchyma elements are present in the wood of a typical *binervosa* root, and some idea of the nature of the xylem may be obtained from Text-fig. 10. Medullary rays are absent except



TEXT-FIG. 9. Transverse section of a root of *S. binervosa*. pxy. = protoxylem; ph. = phloem; per. = pericycle; end. = endodermis; l. = large cells of the cortex; pl. = suberized piliferous layer. $\times 300$.



TEXT-FIG. 10. Transverse section of part of the xylem of the root of *S. binervosa*. Main bank plant. $\times 236$. v. = vessels; f. = fibres.

in the region of the exit of a root-trace, where a multiseriate ray is developed.

Annual rings are usually well marked (cf. Text-fig. 11). The secondary phloem is small in amount, and shows no features of special interest.

Simultaneously with the beginning of secondary growth, the pericyclic cells become meristematic, and the cambium thus produced gives rise to a periderm on its outer margin, thus cutting off the endodermis and cortex.

This periderm is composed of three to four layers of thickish walled cells with dense homogeneous contents. The contents are of the nature of tannin, and may possibly be a Phlobaphene—a decomposition product of plumbagin—the substance stated to occur in the roots of the Plumbaginaceae. Practically no information could be obtained as to the nature of plumbagin, which is described by Tunmann¹ as 'ein wenig erforschter

¹ Tunmann, O.: Pflanzenmikrochemie. Berlin, 1913.

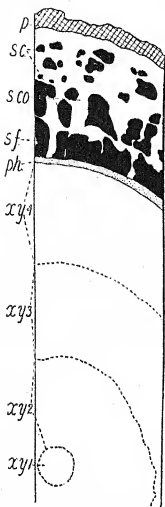
Pflanzenstoff, der möglicherweise in der Zelle in glykosidischer Bindung auftritt', and which he states occurs 'im Zellinhalte in allen parenchymatischen Zellen (auch Markstrahlen, Phloemparenchyma) der Wurzelrinde'.

Examination of the root for the presence of tannin indicates that a substance of this nature occurs in practically all the parenchyma cells of the cortex, medullary rays, and phloem, as well as in the cork: hence it is concluded that this substance is probably identical with the plumbagin of Tunmann, more especially since 'at one time it was thought that tannins were substances of a glucosidic nature and occurred in the plant in combination with a carbohydrate complex such as glucose; . . . this is undoubtedly so in some cases'.¹

The cork cambium gives rise internally to a zone of secondary cortex. This cortex is composed of thin walled parenchyma, the majority of the cells of which contain plumbagin; immediately beyond the phloem lies a broad almost continuous band of sclerenchyma fibres (*s.f.*), while groups of sclerenchymatous cells or sclereides of varying sizes (*s.c.*) are scattered throughout the cortex, as is shown in Text-fig. 11.

It is evident that the structure of such a root as has been described is admirably adapted to the conditions under which the plant grows in the shingle of a lateral bank. The comparatively few vessels and the narrow zone of phloem are in relation to the small translocation which is required in the very slow growing plant. The outstanding characters are the great tensile strength given by the predominance of fibres in the xylem, and the incompressibility provided by the sclereides in the cortex: the combination of these two characters produces even in the very slender roots a structure resembling a steel wire.

An examination of the structure of the root of a plant grown from seed in ordinary soil reveals the effect of the habitat on the structure of the root in a very striking way. In Text-fig. 12, A represents a section through the two-year-old root of a Main bank plant, while B is the root of a plant of similar age grown from seed in garden soil. The proportion of stele

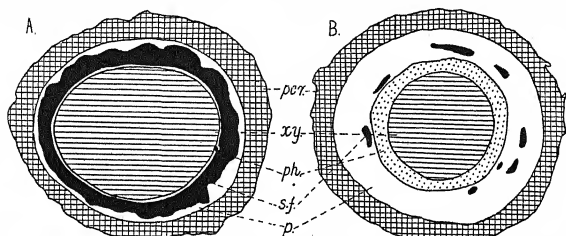


TEXT-FIG. 11. Diagram of part of a root of a Main bank plant of *S. binervosa*. *xy*, 1-4, four annual rings of xylem; *ph*, secondary phloem; *s.f.*, sclerenchyma fibres; *s.c.*, sclereides; *s.c.*, secondary cortex; *p*, periderm.

¹ Haas and Hill: loc. cit., p. 205.

to cortex is markedly different in the two cases. Culture appears to have had the effect of increasing the width of the phloem zone (chiefly by the production of phloem parenchyma), of greatly increasing the amount of cortical parenchyma, and markedly decreasing the proportion of sclerenchymatous fibres present, i. e. culture has considerably reduced the production of incompressibility in the root. Moreover, while parenchyma cells are entirely absent in the xylem of the type plant, in the root of the culture plant the wood is composed of vessels, xylem parenchyma, and very few fibres.

To a much less extent the roots growing in the muddy and sandy-mud lows also show a diminution in the proportion of sclerenchyma and sclereide present in the cortex.



TEXT-FIG. 12. A = diagram of the transverse section of the root of a Main bank plant of *S. binervosa*; B = diagram of the transverse section of a root of a plant of *S. binervosa* cultivated in garden soil from seed. $\times 50$. *xy.* = secondary xylem; *ph.* = secondary phloem; *sf.* = sclerenchyma fibres; *p.* = cortical parenchyma; *per.* = periderm.

Observations which are of some interest in this connexion have been made by van Ufford during the course of his investigation of the Flora of the Pierriers (*Moraine talus*) of the High Calcareous Alps of the Canton de Vaud.¹

The most striking feature in the anatomy of these plants was the development of collenchyma in the peripheral regions of the underground axes. He regards this collenchyma as acting in resisting the thrust of the mobile stones while at the same time permitting without rupture the necessary stretching needed on account of the mobility of the substratum. In the case of *S. binervosa*, since there is no need to provide a mechanical tissue which will allow of further growth in the organ, as the substratum is not mobile, the collenchyma is replaced by the mechanically more efficient sclereides.

Examining the relations between the thickness of the collenchyma and

¹ van Ufford, Q.: Études écologiques de la flore des Pierriers. Thèse. Montreux, 1908.



the radius of the stele, van Ufford invariably found that in closely related plants the more mobile the station the greater the proportion of collenchyma developed. Two examples will suffice to indicate this fact :

<i>Habitat.</i>	<i>Plant.</i>	<i>Thickness of collenchyma radius of the stele.</i>
1. Mobile stones .	<i>Galium helveticum</i>	$\frac{1}{1.5}$
Less mobile earth	„ <i>rotundifolium</i>	$\frac{1}{4.3}$
Fixed earth . .	„ <i>verum</i>	$\frac{1}{6.0}$
2. Mobile stones . .	<i>Cerastium latifolium</i>	$\frac{1}{1.9}$
Less mobile earth	„ <i>arvense</i>	$\frac{1}{2.5}$
Fixed earth . .	„ <i>cuneifolium</i>	$\frac{1}{5.0}$

He further found that only species which have abundant stereome can survive the thrust of the stones ; those which do not possess it die out.

Starr¹ in an examination of the anatomy of dune plants came to somewhat similar conclusions, for she found that comparing the stem of a plant from a mesophytic situation with one of the same species from the dune, the following characters occurred in the dune form :

1. The vessels were more numerous but smaller, the total area being, however, larger.
2. The lumen of the fibres was smaller.
3. The walls of the vessels and fibres were heavier.
4. More sclerenchyma and collenchyma were developed.
5. Slight increase in cork formation occurred.

Finally, Haberlandt thinks that mechanical influences, if they do not pass beyond a certain limit, act on the stereome as a stimulus for further building it up.

An examination of the roots of the various forms of *S. binervosa* from the different habitats shows certain constant differences to obtain :

Annual growth rings. The annual growth rings in the root of the Main bank plant are always wider than in those of a lateral plant ; this is probably in accordance with the less rigorous conditions to which the Main bank plant is subjected. Starr also found a similar relation between plants growing in a mesophytic situation and those growing on a sand dune, and she states, 'a majority of xerophytic forms have more growth rings to the given diameter than the mesophytic forms, showing slower growth under the more adverse conditions.'

Wood parenchyma. Development of wood parenchyma is entirely absent in the plants from the Main bank and the narrow and the broad

¹ Starr, A. M. : Comparative Anatomy of Dune Plants. Bot. Gaz., 1912, vol. liv, p. 265.

leaved lateral plants, except in connexion with the exit of a root-trace. In the plants from the muddy lows the beginning of a year's growth is marked by the formation of wood parenchyma instead of fibres, so that the annual rings are sharply marked off from one another.

In the plants cultivated from seed the xylem shows abundance of parenchyma and very few fibres.

Sclerenchyma and sclereides. The difference in the development of the sclerenchyma ring and the sclereide groups has already been referred to, and is briefly summarized in Table II.

TABLE II. Distribution of the Sclerenchyma Ring and Sclereides in the Various Forms of *S. binervosa*.

Form.	Plant.	Sclerenchyma ring.	Sclereide group.
Tall	Main bank	Broad and almost continuous	Very numerous
	'Narrow-leaved' lateral	" " "	" "
	Muddy low	Narrow and almost continuous	Not numerous
Dwarf	Sandy-mud low	Narrow and discontinuous	Few and small
	Culture from seed	A few isolated fibres only	Very few
! Hybrid	'Broad-leaved' lateral	Broad and continuous, but not quite so broad as in lateral form	Numerous

Vessels. An examination of the vessels in the roots of the various forms shows in every case a gradual increase in size from the primary elements through each successive annual ring (Table III); moreover, in those of the Blakeney forms, in which absorption is presumably more difficult, there is a diminution in the size of the vessels: this is in agreement with the observations of Starr already quoted. The diameter of the vessels in the plants cultivated from seed does not fall into line with the other measurements, and it seems difficult to explain the apparent anomaly.

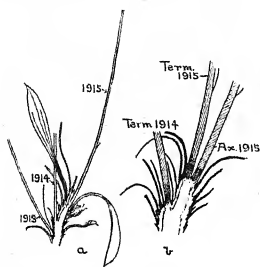
TABLE III. Average Internal Diameter in mm. of the Largest Vessels in the Root and Stem of the Various Forms of *S. binervosa*.

		Dwarf form.					
	Xylem.	? Hybrid form. Broad-leaved.	Tall form. Main bank.	Narrow-leaved.	Sandy.	Muddy.	Culture.
Root.	Primary	0.012	0.010	0.010	0.006	0.006	0.006
	Secondary:						
	Annual ring 1	0.024	0.018	0.014	0.018	0.008	0.010
	" " 2	0.032	0.026	0.020	0.020	0.012	0.014
	" " 3	0.044	0.030	0.024	0.024	0.016	—
	" " 4	—	0.036	0.032	0.028	0.020	—
Stem.	Primary	0.006	0.008	0.006	0.008	0.006	0.006
	Secondary	0.020	0.020	0.016	0.020	0.012	0.010

3. The Stem.

The stem of *S. binervosa* is very short and entirely subterranean, with a much branched crown, each branch bearing a rosette at the ground level. The first inflorescence is terminal and thus brings to an end the growing axis. Growth in the following season is continued by one or more buds developed in the axils of the apical leaves of the

rosette (Text-fig. 13, *a*). In this way the richly branched cushion of the adult becomes formed so that the final condition is an aggregate of closely approximating rosettes. The richly branched crown serves as a trap for sand and silt in which it becomes completely embedded. As the plant increases in size and vigour it will be noticed that from the centre of some of the rosettes more than a single scape is produced. Sometimes this appears to be the result of forking of the scape from its extreme base, but more usually is due to the precocious development of one or sometimes two axillary buds of the terminal leaves (Text-fig. 13, *b*). In these circumstances, if no additional axillary buds develop, the life of the branch ends with the withering of its leaves. The main stem is usually small in diameter, though in a good-sized Main bank plant it may



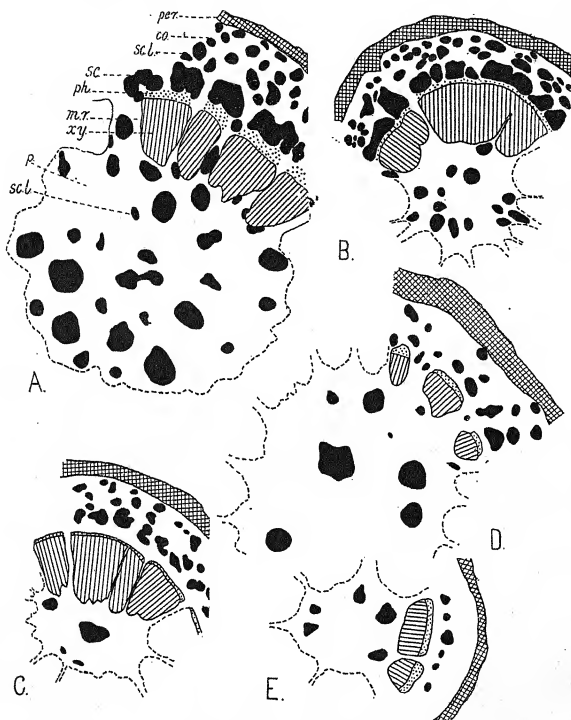
TEXT-FIG. 13. Diagram of the longitudinal section through the apex of rosettes of *S. binervosa*. Explanation in text.

reach as much as 1 cm. across. Text-fig. 14, *A*, shows the arrangement of the various tissues in the stem of a tall form plant. The pith (*p.*) is large, and composed of thin-walled parenchyma, with practically no intercellular spaces; numerous groups of sclereides (*scl.*), varying very much in size, occur in it. Medullary rays (*m.r.*) of thin walled cells break up the vascular ring into numerous segments; the rays vary considerably in width, being often very broad where a leaf trace is making its exit. A group of sclereides is frequently found in the broad rays accompanying the outgoing trace.

The primary vascular strands are very numerous and of small size; secondary growth sets in early, and a narrow zone of secondary wood (*xy.*) composed of vessels embedded in fibrous tissue is produced; annual rings cannot be distinguished in the stem as they can in the root. The elements of the protoxylem have spiral markings, and the rest of the xylem is composed of vessels with simple pits on their walls. The secondary phloem is small in amount (*ph.*). In the pericycle, masses of sclerenchyma fibres (*sc.*) occur opposite to each vascular wedge; no endodermis can be distinguished.

The cortex (*co.*) is made up of small celled, rounded parenchyma, with little intercellular space system; very numerous groups of sclereides (*scl.*) occur in it, their walls are strongly thickened, and they have many branched pits. A broad zone of periderm (*per.*) limits the stem. As in the root, the cells of the periderm and many of the thin-walled cortical cells have contents which are of the nature of tannin (cf. p. 259); this substance is

especially abundant just below the insertion of a leaf, where a double layer of irregular cells indicating the line of union of the broad leaf sheath and the stem is very rich in it.



TEXT-FIG. 14. Diagram of part of stem of *S. binervosa*. A = Main bank plant; B = typical dwarf form; C = broad-leaved form; D = muddy low plant; E = culture plant. per. = periderm; co. = cortex; scl. = scleroides; ph. = secondary phloem; sc. = sclerenchyma fibres; m.r. = medullary rays; xy. = secondary xylem; p. = pith. The dotted line indicates the limit of the pith. $\times 30$.

The stems of plants from the various habitats are in essential features similar to that of the tall form, and only differ in those characters such as have been described for the root, viz. in the proportion of the mechanical tissue present and in the size of the vessels.

A comparison of A, B, C, D and E in Text-fig. 14 shows that the amount of sclerenchyma and the number of sclereide groups diminish considerably in the mud and culture plants as compared with the main bank form and the narrow-leaved lateral one, while the broad-leaved plant shows less marked differences, though the stereome is distinctly reduced.

In the xylem of the sand and mud plants there is a little less fibrous tissue, and slightly more wood parenchyma is developed.

In the culture plant no fibres occurred in the xylem of a two-year old plant, and in a narrow-leaved lateral of a similar age the wood was also free from fibres; but in the lateral plant the wood was much more compact than in the culture form, in which considerable wood parenchyma was present.

Culture, as in the case of the root, has increased the relative width of the phloem zone.

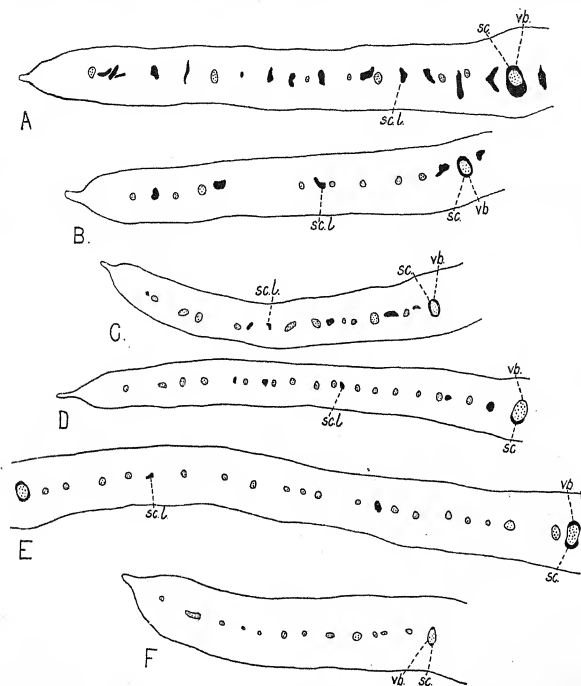
Finally, differences occur in the size of the vessels (see Table II), but since only two-year-old culture plants were obtainable the measurement of the secondary vessels of this stem should not be compared with those of other plants, for increasing age probably means increasing diameter of the vessels up to a certain extent.

These variations in the different forms are in entire agreement with those found in the root, and since the short stem is completely subterranean they are explicable on the same grounds.

4. *The Leaf.*

The general distribution of the tissues in the leaf of the narrow-leaved *binervosa* is shown in Text-fig. 15, A. Numerous small vascular bundles are present, and the mid-vein is encircled by a sclerenchyma sheath (*sc.*); a large number of sclereide groups varying in size occur, and form a characteristic feature of the leaf. Comparison of the proportion of stereome present in the leaf blade of a narrow-leaved plant from the lateral banks and plants from the other habitats shows that in the sand form the sclerenchyma sheath round the mid-vein is much less developed, and the number of sclereide groups distinctly fewer (Text-fig. 15, B); the mud form shows similar features (Text-fig. 15, C); and they are still further emphasized in the plants from the experimental area (Text-fig. 15, D). - The broad-leaved form has the sclerenchyma sheath confined to a cap above and below the main veins, and the sclereide groups are very few in number (Text-fig. 15, E), features which are interesting in comparison with the leaf of *S. bellidifolia* (Text-fig. 15, F), in which also there are only sclerenchyma caps to the main vein, and no sclereide groups at all. Comparison of Text-fig. 15, A, E and F, clearly demonstrates the intermediate character of the putative hybrid and the two suggested parents.

An examination of the stereome in the petiolar region further illustrates the effect of environmental conditions on its development (Text-fig. 16). The petiole of the broad-leaved form (B) is clearly intermediate

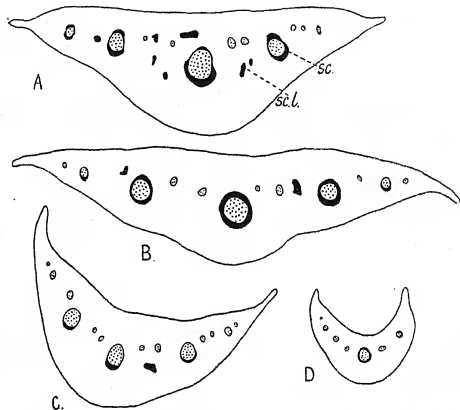


TEXT-FIG. 15. Diagram of part of the leaf blade of *S. hinervosa* (A-E) and *S. bellidifolia* (F) in its middle region, showing the distribution of the stereome. $\times 30$. A = narrow-leaved plant; B = sand plant; C = mud plant; D = plant from experimental area; E = broad-leaved form. vb = mid-vein; sc = sclerenchyma sheath; scL = scleroides. Vascular bundles dotted; stereome black.

between the narrow-leaved plant (A) and *S. bellidifolia* (D). In the experimental plant there is little difference in the proportion present, but, as in the case of the root and stem, culture greatly diminishes the development of mechanical tissue (C). Not only does culture with its more favourable conditions diminish the proportion of stereome present,

but it also has a marked effect on the thickness of the walls of the elements, as can be seen on comparison of A with C and B with D in Text-fig. 17. This again is in entire agreement with the results obtained by Starr in her comparison of mesophytic and dune forms (see p. 262).

In the leaf sheath of all the forms the stereome increases very considerably in amount; the walls of the elements become much thicker, and their length increases greatly; the sclereids become long, fibre-like elements (cf. Text-fig. 17, B and D = typical petiole sclereids, with Text-fig. 18, A and B = typical sheath sclereids).



TEXT-FIG. 16. Diagram of the transverse section of the middle region of the petiole of *S. binervosa* (A-C) and *S. helidifolia* (D). A = narrow-leaved plant; B = broad-leaved plant; C = narrow-leaved plant cultivated from seed. $\times 30$. Stereome in black; vascular bundles dotted; *sc.*, *l.* = sclereids; *sc.* = sclerenchyma sheath.

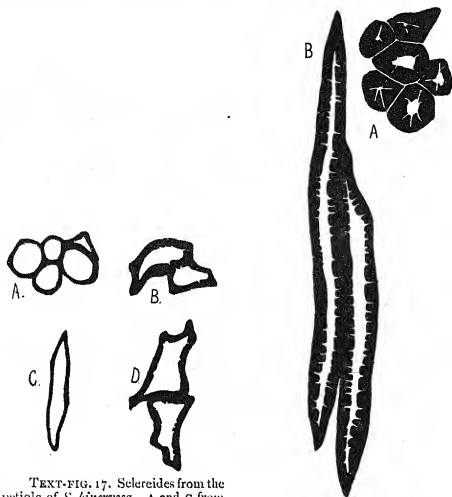
The detailed structure of a narrow-leaved form growing on a lateral is shown in Text-fig. 19, A: on the upper surface is a well-marked palisade layer two or three cells deep; these pass over gradually into the rather shorter palisade-like cells of the lower surface; practically no intercellular spaces are developed. Chlorophyll occurs in all the cells except those of the epidermis: where red coloration is present it is due to red cell-sap. The leaf of a Main bank plant is precisely similar in all respects to that of a narrow-leaved lateral one.

The leaf of the mud plants is on an average slightly thicker than that of the typical form, the increased thickness being due to elongation

of the palisade cells; the average thickness is 0.488 mm. as compared with 0.448 mm. in the narrow-leaved lateral plant.

The sand plants show slight differences in the development of the palisade, only two layers of which are present on the upper surface, and usually only one on the lower, but the individual elements are deeper than in the typical form (Text-fig. 19, B).

Culture exercises a marked influence on leaf structure, for the plants



TEXT-FIG. 17. Sclereides from the petiole of *S. binervosa*. A and C from culture plant; B and D from a narrow-leaved lateral plant. A and B = transverse sections; C and D = longitudinal sections. $\times 170$.

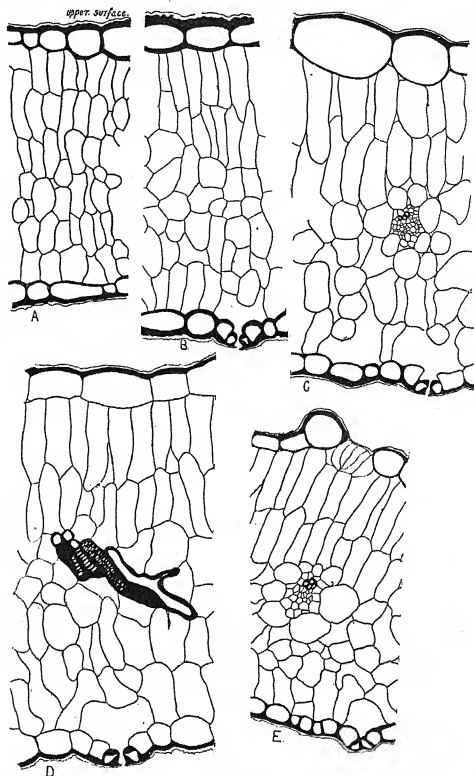


TEXT-FIG. 18. Sclereides from the leaf sheath of *S. binervosa*, narrow-leaved plant. A, transverse section; B, longitudinal section. $\times 220$.

grown from seed have a bifacial leaf, while those at Blakeney are isobilateral. As is shown in Text-fig. 19, C, the palisade on the upper surface is only two layers deep, and the spongy tissue with large intercellular spaces is well developed.

This development of the spongy mesophyll in the culture form is undoubtedly to be related to the absence of the complex of factors acting on the shingle plants. The chief of these are the heat radiation from the hot surface of the shingle during the summer months, and the restricted water-supply of the habitat; the removal of these allows of the production

of a typical bifacial leaf form. Also under cultural conditions the leaves tend to be more erect in position.



TEXT-FIG. 19. Transverse sections of part of the leaf blade of *S. binervosa* (A-D. $\times 235$), and of *S. bellidifolia* (E. $\times 220$). A, narrow-leaved lateral plant; B, sand plant; C, culture from seed plant; D, broad-leaved form; E, *S. bellidifolia*.

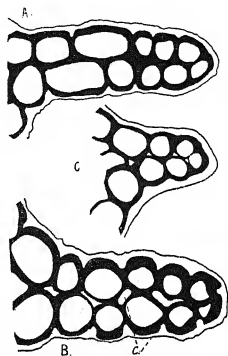
Comparison of Text-fig. 19, A, D and E, again brings out the intermediate character of the leaf of the broad-leaved plant, for both it and *S. bellidifolia* are bifacial in type.

The margin of the leaf of the various forms shows very little variation, for it is always two cells in thickness; the cells have very thick walls, and the angles between them are filled with a substance which gives the reactions of cuticle (Text-fig. 20, B, c). Comparison of Text-fig. 20, A, B and C, shows that the margin of the broad-leaved form is thicker than that of the narrow-leaved, though otherwise it resembles it, and is different from the somewhat blunt margin of *bellidifolia*. This difference between the broad and narrow leaved forms is obviously merely related to the difference in leaf thickness. In the broad-leaved form the average thickness of the middle region of the leaf blade is 0.464 mm. as compared with 0.448 mm. in the narrow-leaved.

The structure of the epidermis shows differences in the different forms, not only in the thickness of the walls but also in the degree of development of the cuticle and in its striation. Text-fig. 21 shows the structure of a typical epidermal cell in each of the forms examined. Comparison of Text-fig. 21, B, C, E and G, brings out the fact that in the forms where the conditions of life as regards water-supply are most severe, viz. in the narrow (B) and broad (G) leaved lateral plants, in the sand plant (C), and in the experimental plant (E), the epidermal cells are larger and thick walled, and have a heavy ridged cuticle, while a well-marked internal cuticle is present as well in the narrow and broad leaved plants and in the sand plant, but is absent in the experimental form, where the conditions are probably a little more favourable.

The epidermal cells of the mud form (Text-fig. 21, D) were small, heavily cuticularized, and an internal cuticle was also present. Here again adequate water-supply is not available for the roots, owing to the difficulty of water absorption in the salt soil and the open character of the vegetation, which renders the air in the region where the leaves are expanded comparatively dry, and this may account for the epidermal structure.¹

The Main bank plant (A) shows a markedly thinner cuticle than the lateral form, and the internal cuticle is absent. This is possibly in relation to the more adequate water-supply which the plant possesses (cf. p. 244). In



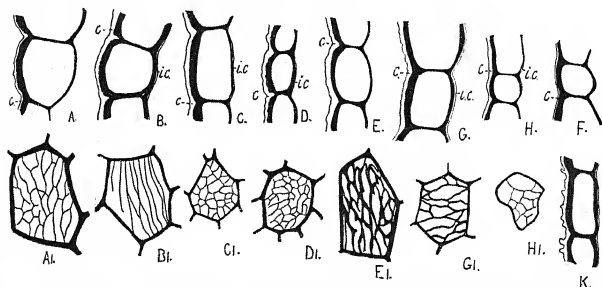
TEXT-FIG. 20. Transverse section of the leaf-margin of *S. binervosa*. Narrow-leaved form (A), broad-leaved form (B), and *bellidifolia* (C); c, cuticle. $\times 250$.

¹ A characteristic feature in the leaves of nearly all salt marsh plants is the very slight development of cuticle, possibly largely owing to the fact that their aerial parts are expanded in a distinctly humid atmosphere.

the culture plant (F) the cuticle is markedly thinner than in the other forms; the epidermal cells are also smaller, and no trace of internal cuticle was seen.

The cells of the lower epidermis show characters essentially similar to those of the upper, except that the ridging of the cuticle is slightly more pronounced in the case of the Main bank, narrow-leaved lateral, and mud forms, while in the sand plant the ridging is much more marked (Text-fig. 21, K), a fact which is probably to be correlated with the reflection from the sandy surface.

The striation of the cuticle as seen in surface view is shown in Text-fig. 21. It varies a little in the different forms, and it is significant that practically none occurs in the culture form.



TEXT-FIG. 21. Upper epidermal cells of *S. binervosa* (A-G), and of *S. bellidifolia* (H). A-H and K in transverse section. $\times 220$; A1-E1, G1, H1, in surface view, showing striation of the cuticle. $\times 168$. A, A1 = Main bank plant; B, B1 = narrow-leaved lateral plant; C, C1 = sand plant; D, D1 = mud plant; E, E1 = experimental plant; F = culture plant; G, G1 = broad-leaved plant; H, H1 = *S. bellidifolia*; K = lower epidermis of a 'sand' plant of *S. binervosa*. c = cuticle; ic = internal cuticle.

Stomata occur on both surfaces of the leaf; they show the constant character of being surrounded by three subsidiary cells (often four in *S. bellidifolia*) (cf. Text-fig. 24, A and C). In all the forms examined they appear essentially similar in structure, and only differ in the fact that those of the Main bank plant are larger than in any other form, though those from the experimental area are nearly equal to them in size: the stomata of the mud form are the smallest, those of all the other plants are approximately equal to those of the narrow-leaved plants. They usually occur on the level of the epidermal cells, though they may be very slightly raised above them.

The number of stomata on the two sides of the leaf is approximately the same, but differences occur in the different forms, as is shown in

Table IV; comparison with Table I shows that, generally speaking, increase in the number of glands corresponds with increase in the number of stomata :—

TABLE IV. Average Number of Stomata on the Leaf Blade in *S. bellidifolia* and the Various Forms of *S. binervosa*, per sq. mm. of Area.

Form.	Plant.	Upper surface of leaf blade.	Lower surface of leaf blade.
? Hybrid	<i>S. bellidifolia</i>	73.3	35.9
	Broad-leaved <i>binervosa</i> . . .	42.1	35.2
	<i>S. binervosa</i> :		
Dwarf	muddy low	63.6	63.6
	sandy-mud	56.0	51.2
	culture from seed	37.3	35.2
	narrow-leaved lateral	33.2	26.3
	experimental	36.6	44.2
	binervosa zone of a lateral (one year in greenhouse)	32.5	35.2
	crest of a lateral (one year in greenhouse)	16.6	23.5
Tall	<i>S. binervosa</i> , Main bank plant	34.6	31.8

The structure and distribution of the mucilage and Mettenian glands has already been fully considered (see pp. 251-7).

The Mettenian are generally sunken slightly below the epidermal level, while the mucilage glands are raised slightly above it. In the leaves of the culture plant, however, the Mettenian glands are level with the surface. In the broad-leaved form the epidermal cells surrounding a gland are somewhat enlarged, a feature which recalls *S. bellidifolia*, where they are enormous when compared with the remaining epidermal cells.

The distribution of tannin in the leaf blade is similar in all the forms. The layers of cells immediately adjacent to the epidermis, both upper and under, are very rich in a substance giving the reactions of tannin (see p. 259), and these two layers contain very little starch; the two layers immediately internal to them are very rich in starch and contain practically no tannin. Many of the remaining mesophyll cells have tanniferous contents, more especially those in the neighbourhood of the vascular bundle of the midrib. In the petiole this substance occurs in the subepidermal layer and in numerous isolated parenchyma cells. Enormous quantities are present in the mud plant.¹

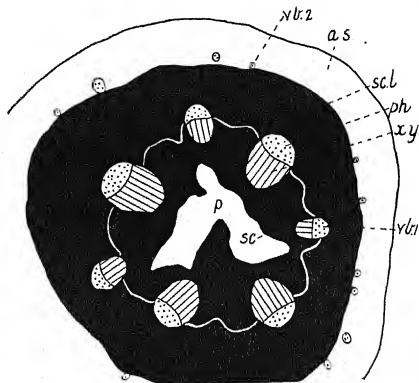
5. The Inflorescence Axis.

The distribution of the tissues in the inflorescence axis is shown in Text-fig. 22. The pith (*p.*) is surrounded by a ring of large, closed, collateral bundles (*vb.1*). In the lower parts of the axis in the pith one or more bundles of sclereides may occur, but they usually die out before the insertion of the first scale leaf. The number of vascular bundles varies greatly; it depends on the size of the inflorescence and the degree of branching; the number decreases after the emission of each branch. The

¹ For the probable function of the tannin see pp. 253, 259-60.

vascular strands are embedded in a mass of lignified tissue (*sc.*), the outer layers of which are sclerized (*sc.l.*) in the stouter inflorescences. In the smaller axes this sclerized zone becomes reduced and finally disappears, and the inner ring of bundles are no longer completely embedded in fibres.

Immediately beyond the sclerized zone is a ring of very small bundles (*vb.2*); they lie at the margin of the assimilating region of the axis (*a.s.*). Below the level of insertion of the first scale leaf these small bundles join on to the internal ring of larger strands. The assimilating layer is composed of about four rows of small, rounded, parenchyma cells, but above



TEXT-FIG. 22. Diagram of part of the inflorescence axis of *S. binervosa*. $\times 70$. *a.s.* = assimilating cortex; *vb.1* = inner ring of bundles; *vb.2* = outer ring of bundles; *sc.l.* = sclerized zone; *sc.* = lignified tissue; *ph.* = phloem; *xy.* = xylem. The white line indicates the limit of the sclerized zone.

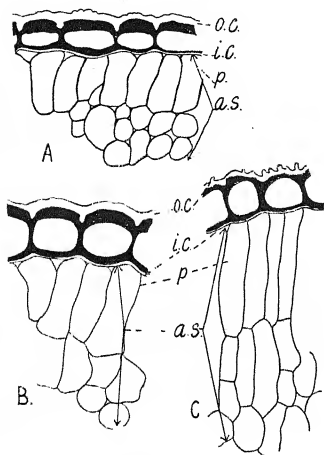
the insertion of the scale leaf the outermost layer of these cells becomes palisade-like (*p.*, Text-fig. 23, A), while in the ultimate branchlets two layers of palisade cells are present.

The epidermal cells have very thick walls, especially on the outer surface; the external cuticle (*a.c.*) is heavily developed, with a ridged outer surface and plugs between the cells; a well-marked internal cuticle also occurs (*i.c.*, Text-fig. 23, A). Stomata and Mettenian glands are both present on the inflorescence axis.

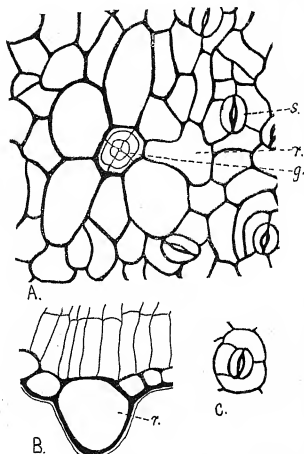
The general structure of the inflorescence axis is the same in all the forms of *S. binervosa*, and it is only in the broad-leaved plant that any noteworthy variations occur. The palisade layer of the flowering spikes of

this plant consist of one long and one shorter layer of cells (Text-fig. 23, B), and it is intermediate in this respect between its possible parents (cf. Text-fig. 23, A, B and C).

It is in the surface of the axis, however, that the chief difference occurs. In the tall and dwarf forms of *binervosa* it is quite smooth, except in the ultimate branchings. In the ? hybrid all the branches are slightly rough, but in *bellidifolia* the scape is distinctly granulated throughout. The rough



TEXT-FIG. 23. Part of the inflorescence axis of *S. bellidifolia* (C), narrow-leaved *binervosa* (A), broad-leaved *binervosa* (B) (taken from comparable levels). $\times 347$. o.c. = external cuticle; i.c. = internal cuticle; p. = palisade; a.s. = assimilating layer.



TEXT-FIG. 24. A. Gland in surface view from the inflorescence axis of *S. bellidifolia*. $\times 293$. B. Part of the epidermis showing one of the rosette cells. $\times 220$. r. = rosette of enlarged cells; g. = gland; s. = stoma with four subsidiary cells; C. Stoma of *S. binervosa* with its three subsidiary cells. $\times 165$.

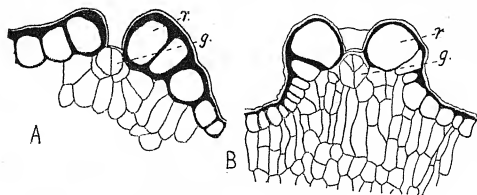
appearance is due to the fact that the epidermal cells surrounding a gland are considerably enlarged and papillate, resulting in the sinking of the gland in a depression, while the glands in surface view appear as rosette-like structures. This is clearly shown in Text-fig. 24, A.

The slight wartiness of the axis of the broad-leaved form is due to a similar, though less pronounced, arrangement of the cells surrounding the gland (cf. A and B, Text-fig. 25).

In all the other forms of *binervosa*, though an occasional enlargement

of the cells may occur in the ultimate branches of the scape, it is never developed as a constant character.

In the barren spikes of the broad-leaved *S. binervosa* the assimilating tissue is much more developed; the palisade cells are longer and are three



TEXT-FIG. 25. Transverse section of part of the inflorescence axis of broad-leaved *S. binervosa* (A), and of *S. bellidifolia* (B), showing the difference in the projection of the gland areas. $\times 167$. g. = gland; r. = 'rosette' cell.

deep, a feature which recalls the barren scapes of *S. bellidifolia*. The vascular bundles are also small, and the sclerized zone is only feebly developed.

STATICE BELLIDIFOLIA.

The habitat of this plant at Blakeney Point has been described on p. 244. Only plants from the lows were available for investigation.

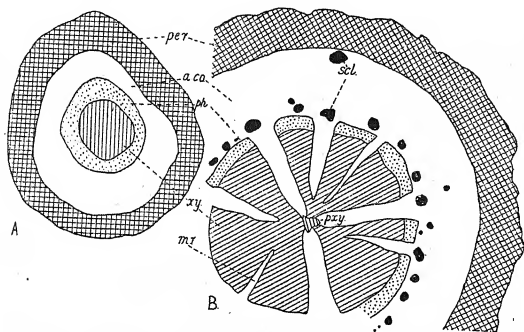
1. The Root.

The structure of the root differs essentially from that of *S. binervosa*, and much more closely resembles that of the salt marsh forms such as *S. Limonium*; it is much less wiry and distinctly more fleshy. The distribution of the tissues in the root is shown in Text-fig. 26, A; a comparison with Text-fig. 12, A, shows the very different proportions which obtain between the mechanical tissues in *bellidifolia*, which is essentially a salt marsh plant, and *binervosa*, which inhabits shingle.

The primary root has a diarch or tetrarch plate of xylem; secondary growth sets in early and produces considerable secondary wood (xy., Text-fig. 26, A) surrounded by a broad zone of secondary phloem (ph.). The secondary xylem is composed of comparatively few large vessels embedded in xylem parenchyma; occasional wood fibres occur, but the wood is distinctly 'soft' in type, and very different in nature from that of *binervosa* (cf. Text-fig. 27 and Text-fig. 10). Broad primary medullary rays occur opposite the protoxylem groups, and wide secondary rays split up the wood into segments and still further increase its parenchymatous nature.

A broad zone of secondary phloem (ph., Text-fig. 26, A) is produced; the bulk of it is phloem parenchyma. The secondary cortex is a wide zone of

acrating tissue (*a.co.*), precisely similar to that of *Salicornia*,¹ or of the salt marsh species of *Statice*. This aerenchymatous cortex is in obvious relation to the habitat of the plant in lows, the soil of which must often be water-logged for considerable periods. A broad zone of periderm encircles the cortex. Throughout the greater part of the root no sclerenchyma or sclereids occur, but near the junction with the stem nests of sclereids develop mostly in a single series immediately beyond the phloem zone (*scl.*, Text-fig. 26, B).

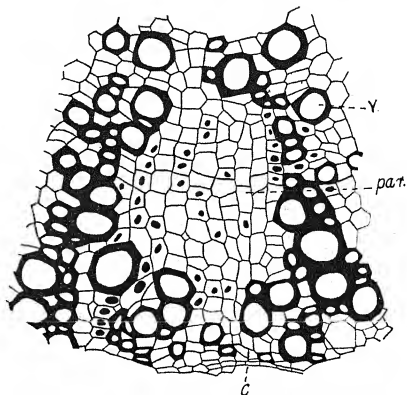


TEXT-FIG. 26. Diagram of a transverse section of a root of *S. bellidifolia*. A = young root. $\times 43$; B = old root near its junction with the stem. $\times 60$; *pxy.* = protoxylem; *xy.* = secondary xylem; *ph.* = secondary phloem; *a.co.* = acrating cortex; *m.r.* = medullary ray; *scl.* = sclereids nest; *per* = periderm.

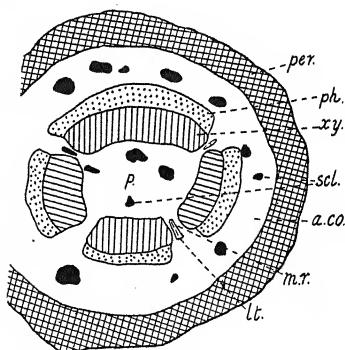
2. The Stem.

The stem of *S. bellidifolia* is short and more completely embedded in the mud than in *S. binervosa*. The distribution of the tissues is shown in Text-fig. 28. The pith (*p.*) is large, and contains nests of sclereids (*scl.*), though they are less abundant than in *S. binervosa* (cf. Text-fig. 14, B). The vascular bundles are broken up into a few large wedges by very wide medullary rays (*m.r.*), which only occur at the point of exit of leaf-traces (*lt.*). The xylem contains comparatively few vessels, but numerous fibres; the size of the vessels and their markings are the same as those of the Main bank *S. binervosa* (see Table III, p. 263). The zone of secondary phloem (*ph.*) is wide, and consists chiefly of phloem parenchyma. The cortex, in which, as in the pith, the intercellular space system is well developed, shows no sclerenchyma and only a single series of sclereid groups, and the zone

¹ de Fraine, E.: The Anatomy of the Genus *Salicornia*. Journ. Linn. Soc. Bot., 1913, vol. xli, p. 337.



TEXT-FIG. 27. Detail of part of xylem in root of *S. bellidifolia*. *c.* = cambium; *par.* = wood parenchyma; *v.* = vessel. $\times 290$.



TEXT-FIG. 28. Transverse section of the stem of *S. bellidifolia*. $\times 40$. *p.* = pith; *xy.* = secondary xylem; *ph.* = secondary phloem; *m.r.* = medullary ray; *lt.* = leaf-trace; *scl.* = sclereide group; *a.co.* = aerating cortex; *per.* = periderm.

of periderm is very wide. In the nature and proportion of the stereome present, the stem approximates very closely to the stem of the plants of *binervosa* cultivated from seed (cf. Text-fig. 28 and Text-fig. 14, E); indeed

practically the only difference between the two lies in the greater development of air-spaces in *bellidifolia* and the greater width of the cork layer.

The distribution of tannin is similar in the species to that of *S. binervosa*.

3. The Leaf (cf. p. 249).

The general structure of the leaf is shown in Text-fig. 15, F; it is distinctly fleshier than in any of the forms of *S. binervosa*, and is at once distinguished from them by the absence of sclereides in the blade and petiole, though they occur in the leaf sheath; sclerenchyma fibres are also very few in number, and are present only as a small cap above and below the main veins. The details of the leaf and petiole structure are shown in Text-figs. 15 D, 19 E, 20 C, 21 H, and 21 H I; they have been described on pp. 266-73 in comparison with the leaves of *S. binervosa*.

4. The Inflorescence Axis (cf. p. 250).

The general structure of the inflorescence axis resembles that of *S. binervosa* (see pp. 273-6), but the number of bundles in the inner ring is usually much fewer. As in *S. binervosa*, frequent anastomoses take place between the small cortical strands and also between the bundles of the inner and outer ring. The main bundles of the scale leaves are furnished from one of the inner ring of bundles, and the remaining bundles are provided by the cortical strands. Details of the structure of the axis are given on pp. 273-6, and are illustrated in Text-figs. 23 C, 24 A and B, and 25 B.

IV. COMPARISON OF THE ANATOMY OF THE BROAD-LEAVED *S. BINERVOSA* AND ITS POSSIBLE PARENTS.

	Narrow-leaved <i>S. binervosa</i> = A.	<i>S. bellidifolia</i> = B.	? Hybrid, broad-leaved <i>S. binervosa</i> = C.
Root:	(12 A: 10)	(26)	
Type	Shingle	Marsh	Shingle
Periderm	Present	Wider zone than A	Present
Cortex	Not aerating	Aerating	Not aerating
Sclerenchyma and sclereides	Abundant	Absent throughout the greater length of root	Less abundant than in A
Phloem	Very narrow zone	Wide zone	Intermediate between A and B
Xylem:			
Fibres	Numerous	Few	Numerous
Wood parenchyma	Absent	Abundant	Absent
Primary vessels	0.010 mm. diameter	0.006 mm. diameter	0.012 mm. diameter
Secondary vessels	0.020 mm. diameter	0.028 mm. diameter	0.032 mm. diameter

	<i>Narrow-leaved</i> <i>S. binervosa</i> = A.	<i>S. bellidifolia</i> = B.	† <i>Hybrid, broad-leaved</i> <i>S. binervosa</i> = C.
<i>Stem</i> :	(14 B)	(27)	(14 C)
Type	Short and subterranean	Short and subterranean	Short and subterranean
Periderm	Wide	Wider than A	Wide
Cortex	Not aerating	Slightly aerating	Not aerating
Sclerenchyma and sclereides	Very abundant	Very few	Abundant
Phloem	Narrow zone	Wide zone	Narrow zone
Medullary rays	Narrow	Wide	Narrow
<i>Xylem</i> :			
{ Fibres	Numerous	Numerous	Numerous
{ Primary vessels	0.006 mm. in diameter	0.006 mm. in diameter	0.008 mm. in diameter
{ Secondary vessels	0.016 mm. in diameter	0.020 mm. in diameter	0.020 mm. in diameter
{ Pith	Many sclereides	Few sclereides	Intermediate between A and B
<i>Leaf</i> :			
Type	Isobilateral (19 A)	Bifacial (19 B)	Bifacial (15 D)
Thickness	Thin. Average = 0.448 mm.	Thicker than C	Intermediate between A and B. Average = 0.464 mm.
Sclereides in blade	Abundant (15 A)	Absent (15 F)	Few (15 E)
Sclerenchyma	Much	Little	Intermediate between A and B
Margin	Long and narrow (20 A)	Short and broad (20 C)	Like A, but thicker (20 B)
<i>Cuticle</i> :	(21 B, B 1)	(21 H, H 1)	(21 G, G 1)
{ External	Thick, ridged, plugs between the cells	Thick, less ridged, no plugs	Thick, ridged, plugs present
{ Internal	Present	Traces only. Epidermal cells smaller than in A and C	Present
<i>Glands</i> :			
{ Mucilage	Abundant	Less abundant	Abundant
{ Mettenian	Upper surface, 6.2 per sq. mm.	9.6	8.3
	Lower " 8.9 " "	8.3	9.6
<i>Stomata</i>	Upper surface, 33.2 per sq. mm.	73.3	40.1
	Lower surface, 26.3 per sq. mm.	35.9	35.2
<i>Petiole</i> :			
Type	Flat (16 A)	Channelled (16 D)	Flat (16 B)
Sclerenchyma	Abundant	Little	Less abundant than in A
Sclereides	Numerous	Absent, except in the sheath	Less numerous than in A
<i>Inflorescence axis</i> :			
Surface	Smooth	Very granulated (24 A, B; 25 B)	Slightly granulated (25, A)
Palisade layer	One layer (23 A)	Two layers (23 C)	Two layers (23 B)

(The numbers and letters in round brackets indicate the diagram illustrating the character in question.)

V. SUMMARY.

1. *The morphology and anatomy* of the forms of *S. binervosa* and *S. bellidifolia* which occur at Blakeney Point, Norfolk, is described. Three main forms of *binervosa* are distinguished:

(a) The tall form which grows on the edge of shingle fans on the Main bank (pp. 241 and 244).

(b) The dwarf form characteristic of stable lateral banks (pp. 240-1) (the typical narrow-leaved form), but which also occurs on the margin of muddy lows (mud plants), and of sandy-mud lows (sandy plants).

(c) The ? hybrid form between *S. binervosa* and *S. bellidifolia* (p. 245).

The habitats of all the forms are described, and the ecological factors involved and their possible effects on the plants are considered. Experimental evidence is brought forward to show the temporarily stimulating effect of shingle on growth—the effect is due to mulch action (pp. 240-6).

2. Glands (pp. 251-7).

The structure, number, and distribution of the two kinds of glands characteristic of the order are described. The mucilage glands function in preventing desiccation of the apex; the Mettenian glands (the so-called chalk glands) excrete water, but probably only function when the amount absorbed by the roots is greater than the rate of transpiration.

Some explanation of the numerical differences which occur is given.

3. The Seedling (pp. 257-8).

The morphology and the seedling structure of *S. binervosa* is described; the method of the transition from root to stem follows van Tieghem's Type 3.

4. Root (pp. 258-63 and 276-7).

The structure of the root in all the forms, and of the plants cultivated from seed, is described. In *S. binervosa* the structure is in every way adapted to withstand the pressure of shingle and is admirably suited, both internally and externally, to life in an habitat characterized by scarcity of water. The root of *S. bellidifolia*, on the other hand, resembles that of many salt marsh plants, and shows none of the characters of stabilized shingle plants. Differences between the roots of the two species are chiefly seen in the following features:

(a) The proportion and distribution of the stereome-sclerenchyma and scleroides.

(b) The nature of the xylem elements.

(c) The character of the cortex.

The effects of the differences in the habitats of the various forms of *S. binervosa* are considered; the chief variations occur in:

(a) The annual growth rings.

(b) The development of wood parenchyma.

(c) The proportion of sclerenchyma and scleroides.

(d) The size of the vessels.

5. Stem (pp. 263-6 and 277-9).

The stem in both species is short and subterranean; its outstanding characters are the abundance of fibres in the wood and the nests of scleroides in the pith and cortex. The differences obtaining in the various

forms of *S. binervosa* are considered; they are of a similar nature to those present in the root and are due to similar causes.

6. *Leaf* (pp. 256-73 and

The structure of the leaf is bifacial in *S. bellidifolia* and in the hybrid *S. binervosa*, but is isobilateral in all the other forms. Differences occur in the abundance of the sclereides present; they are numerous in the typical *binervosa*, absent except in the leaf sheath in *bellidifolia*, and few in the ? hybrid. The effects of the various habitats on their production is examined, and the influence of culture under favourable conditions in diminishing mechanical tissue (as in the root and stem also) is pointed out.

The effect of culture on the development of the intercellular space system, and on the production of cuticle, indicates a definite reaction of the plants to the external conditions. The degree of development of the cuticle, both internal and external, appears to depend on the water relations of the habitat in all the forms examined.

The stomata have three subsidiary cells in *S. binervosa* and four in *S. bellidifolia*; they occur on both surfaces of the leaf, and details of their distribution are given.

7. *Inflorescence axis* (pp. 273-6 and 279).

An inner series of large, collateral bundles and an outer series of small cortical ones occur in the axis. The inner bundles are embedded in lignified fibres and are surrounded by a zone of sclereides. The cortical bundles lie at the margin of a narrow assimilating zone, at the outer edge of which is a palisade layer of one (dwarf forms of *binervosa*) or two cells (? hybrid *binervosa* and *bellidifolia*). In the sterile branches of *bellidifolia* and the ? hybrid *binervosa* the palisade cells become deeper and the number of layers is increased.

The proportion of stereome diminishes in the smaller axes.

The surface of the inflorescence axis is smooth in *S. binervosa*, slightly rough in the ? hybrid form, and distinctly scabrid in *S. bellidifolia*; the roughness is due to the enlargement of the cells surrounding the Mettenian glands.

8. The anatomical characters of the ? hybrid and its two parent forms are summarized (pp. 279-80).

9. The floral morphology of *S. binervosa* and the ? hybrid form, and of *S. bellidifolia*, is fully described (pp. 246-50).¹

It is a great pleasure to acknowledge my indebtedness to Professor F. W. Oliver, both for the help he has given me in connexion with the experiments and also for specimens of plants collected at various times.²

¹ The author is entirely indebted to Dr. E. J. Salisbury for this section of the paper.

² The investigation was partly carried out in the Ecological Laboratory at Blakeney Point, Norfolk.



FIG. 1. Dwarf form of *Statice binervosa*.

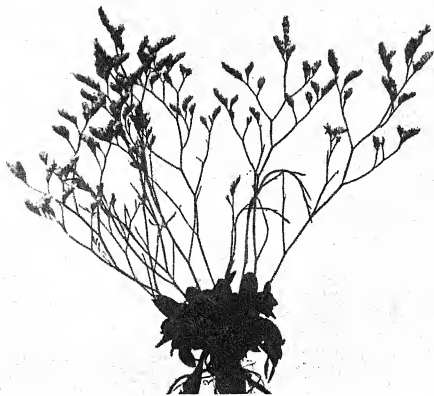


FIG. 2. Broad-leaved variety of *Statice binervosa* (? hybrid *S. binervosa* x *S. bellidifolia*).

DE FRAINE — STATICE.



Studies of Protoplasmic Permeability by Measurement of Rate of Shrinkage of Turgid Tissues.

I. The Influence of Temperature on the Permeability of Protoplasm to Water.

BY

E. MARION DELF,

Yarrow Fellow, Girton College, Cambridge.

With seventeen Figures and five Tables in the Text.



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INTRODUCTION.

THE effect of temperature on the permeability of protoplasm to water has much interest biologically, but no critical work dealing with it has hitherto been published. It was known that, generally speaking, root absorption is retarded by low soil temperatures, and Wieler states that the bleeding of vine stems is accelerated eight times by a rise in temperature from 8° to 42° C. The first attempt to measure the temperature effect was the work of Krabbe in 1895, and was based on the plasmolysis of cylinders of turgescient pith tissues at different temperatures, the time required for the total contraction in any given solution varying with the temperature. This method was adopted by van Rysselberghe in 1901, and extended to the consideration of the passage of water through the protoplast both in plasmolysis and deplasmolysis. According

to van Rysselberghe, the effect of temperature is accelerating only up to 20°C ., but this conclusion is open to criticism both on experimental and on theoretical grounds, as will be shown in the course of this paper.

It was suggested to me by Dr. F. F. Blackman that the plasmolytic method of investigating the effect of temperature on the permeability of protoplasm to water could be made more effective by the use of some magnifying arrangement enabling the observer to follow the process in all its stages. This has been achieved by means of a particular form of optical lever devised by him, which gives a magnification of 350 diameters on changes in length of short strips of tissue, which though fixed in position are not subjected to any strain. I am indebted to Dr. Blackman not only for the use of this apparatus, but for much help and suggestion throughout the research. The work was carried out in the Cambridge Botany School in the year 1915, while holding a Yarrow Research Fellowship at Girton College.

SECTION II. APPARATUS AND REGULATION OF TEMPERATURE.

The method of measuring the rate of tissue-shrinkage is based upon the great magnification of alterations of length of strips of tissue that can be obtained by the principle of the 'optical lever'.¹ The short lever C, which the plant actually displaces, carries a tiny mirror on which the image of a cross wire is projected by a Nernst burner (v, Fig. 1) in a tube. From the mirror the image is reflected on to a millimetre scale W, and a magnification of $\times 350$ is thus attained. The fulcrum of the optical lever is a small aluminium cylinder free to rotate on a horizontal axis, the bearings being steel points working in agate cups. From the cylinder adjustable wires project at right angles to the axis for a few mm., and to these the threads connecting to the plant tissue below on one side and to the counterpoise D below on the opposite side are attached. The counterpoise is a few mm. of fine wire and so adjusted that it will just assure a downward movement of its own side when the thread connecting to the plant on the other side is cut. There is thus always a minute extension-strain on the plant tissue. The clamp for the plant below is carried on the same 'invar' rod, E, as the lever, and the whole is supported from above independently of the rest of the apparatus.

The clamp is designed to hold a tubular structure such as part of an inflorescence axis or a cylindrical leaf, so that water or solution may be passed through it continuously, and bathe the delicate pith cells which line the interior. The plant cylinder A (Fig. 1) is firmly fixed to a glass nozzle G of narrow bore, by winding it round with cotton fastened below to a small rectangular block of cork through which the nozzle passes. The

¹ The principle of the optical lever has been previously used by Professor Bosc for records of minute movements of plants; see J. C. Bosc: *Plant Response*, 1906.

a cork in the neck of the flask, two of which are governed by the three-way tap at K, whilst the third, I, is an outflow, the level of which at M determines the rate of flow of the liquid through the chamber and coil; the side tube O is an additional outlet for more rapidly emptying the chamber.

By means of the tap K a liquid entering through Z can be diverted at will to the tube leading to the coil H or to the tube J which acts as a by-pass; or the tap can be turned so that no liquid can enter from Z. The whole flask is packed in cotton-wool or ice for high and low temperatures respectively, and is encased in a wooden box to diminish further loss of heat by radiation. The box is raised on a wooden block P during an experiment when the plant cylinder is immersed in water in the conical flask. The block can be removed by a handle when the chamber is to be lowered; the nozzle can then be adjusted or removed, together with its clamp, to receive a new piece of tissue. When an experiment is being made, a thermometer is also suspended in the chamber so that its bulb stands close by the plant cylinder. (In the figure this is shown on the opposite side.)

The flow of liquid entering Z is determined by two three-way taps placed one on either arm of a double Y-tube and connected with delivery tubes x and y leading from the solutions. x and y are further connected with long flat coils of glass tubing (not shown in the diagram), and both are surrounded with a large water-bath, Q, kept at constant temperature by means of a thermostat. A current of water or solution passes from the aspirators along one of the coils where it acquires the temperature of the water-bath. It then enters the tube X or Y and can be diverted at will to either the outer tubes T or S leading to the chamber, or to the inner tubes U or R leading to the outflow N. It is therefore possible to have two streams of liquid running at the same rate, traversing an equal distance through the same water-bath and hence acquiring the same temperature; and either of these liquids can be turned through the plant cylinder immediately without any perceptible change in temperature.

SECTION III. PROCEDURE IN A TYPICAL EXPERIMENT.

The optical lever was first adjusted relative to the source of light so that a sharply defined cross wire in a bright field of light was thrown upon the distant millimetre scale; the lever, light-source, and scale remained in these positions throughout the experiments and together gave a magnification of nearly 350 diameters. This was estimated directly by measuring the deflexion of a spot of light on the scale when a vertical thread attached to the middle arm of the optical lever was depressed (by hanging on it a very small piece of fine wire) through a distance of 1 mm. as measured by a vertical scale immediately behind it.

A small counterpoise attached to the free arm of the optical lever was adjusted by trial until the tension in the plant cylinder, when fixed

in position, was reduced to a minimum. That this was accomplished was evidenced by the fact that even the most flaccid tissues when left attached to the lever showed no sign of the slow creeping extension that an appreciable elongating strain must produce.

Whilst very sensitive to any change in the length of the plant cylinder, the apparatus would give the same reading for many hours when a turgid piece was kept at constant temperature and freely supplied with water. In all the experiments, the freshly gathered material was kept in slowly running tap-water for some hours before use in order to ensure that the material was turgid. In ordinary cases one or two hours in water was sufficient for this purpose, but in very dry weather six hours or even more would be necessary. This previous immersion appeared to have no effect on subsequent treatment with sugar. When first placed on the apparatus distilled water was passed through the plant cylinder to see whether any further intake of water was occurring; the sugar solution was only passed through when the length of tissue showed a practically constant reading with the distilled water.

For an experiment at any temperature other than that of the laboratory the water-bath and chamber were previously adjusted so that the latter was at the desired temperature with distilled water flowing through the apparatus. Several trials were made beforehand, and it was found that, apart from variations in the temperature of the laboratory itself, the temperature of the water-bath had to be well above that desired in the chamber, e. g. from 3° at lower temperatures to 15° C. at highest temperatures.

The control of the temperature was effected by (a) cooling or heating the water-bath, and (b) regulating the rate of flow of liquid through the apparatus. A temperature of 5° to 6° C. was produced in the chamber by packing the water-bath with ice and salt, and keeping melting ice in the chamber; by this means a constant temperature could be maintained for hours. A temperature of 8° to 10° C. was obtained by keeping the water-bath cool with ice and salt, but leaving the chamber to establish its own equilibrium. At high temperatures the water-bath was heated by means of a gas flame regulated by a thermostat, and the rate of flow of the two solutions to be used was carefully adjusted beforehand, so that at a known rate for each solution the temperature of the chamber would be maintained. The regulation of the flow of liquid was achieved by adjusting the pressure under which the liquid was driven through the apparatus, aided by the various taps, which could be turned partly off if necessary. The supplies of liquid were contained in 'aspirators'—Marriotte bottles—which give a constant flow of liquid; so adjusting the height of the exit-tube, x , of the chamber determined the rate at which, other things being equal, the liquid would flow through the apparatus. With practice it became possible to keep the temperature of the chamber constant during

an experiment within 1° C. including the change from water to the plasmolysing solution.

When the chamber temperature was constant, the tap K (Fig. 1) admitting the water current was turned off, the outlet M of the chamber blocked, and the chamber lowered by removing the block of wood P upon which it stood. The metal clamp containing the glass nozzle was removed from its socket, and the piece of tissue was then fitted to the nozzle and bound tightly. The nozzle was then inserted in the clamp, the clamp fixed in position again so that the material was vertical, and the glass hook inserted so that the spot of light from the mirror occupied a convenient position on the scale. The chamber was then raised, and the tissue therefore at once submerged in the water of the chamber at the required temperature. The tap K was at once turned to admit water, the exit-tube M unblocked, and a reading of the scale taken. The whole process of fixing on the plant after the temperature was established was a matter of about one minute, so that there was no unnecessary preliminary exposure of the tissue before the beginning of the observations.

During the course of an experiment, readings of the scale were made at frequent intervals, concurrently, with readings of the thermometer in the chamber. At high temperatures the readings were often taken every half-minute when first the plasmolysing solution was turned through the apparatus.

Just before the sugar solution was to be given to the plant, and whilst the latter was still supplied with water, the sugar current was circulated through the path V, R, N (Fig. 1) and out to a waste receiver. The rate of this current was adjusted until it was equal to that of the water current through the chamber, and in a few minutes its temperature was that of the water leaving the water-bath. The water current was then diverted from the plant to the 'by-pass' J (Fig. 1) by the three-way tap K and the taps of X and V (Fig. 1) reversed so that the paths of the sugar and water currents were interchanged. The sugar reached the chamber tube Z in thirty seconds, and the tap K was turned so that the sugar passed up the coil and through the plant. Immediately after turning the tap K a reading was made of the scale, and in about thirty seconds, when the denser sugar solution could be detected streaming over the edge of the cylinder, a second reading was made. During the replacement of water by solution there was no variation in the position of the spot of light on the scale. In some cases after plasmolysis had been observed, the water current was again turned through the plant, in order to observe the course of deplasmolysis.

SECTION IV. CHARACTERISTICS OF THE MATERIAL USED.

The leaves of onions and the scapes of dandelions were used throughout the experiments, since these are readily obtainable, of suitable diameter, fairly uniform in structure, and not too rigid. In order to ensure as far as possible the supply of comparable material, a number of scapes and leaves were marked and kept under observation. An examination was also made of their structure to see what tissues were mainly concerned in the plasmolytic shrinking. It was clear that in both cases the cavity of the interior is well lined with thin-walled living cells which would be freely exposed to the action of any solution flowing through it.

A. Onion Leaves.

Onion leaves were used from plants in their second season of growth, i. e. from full-grown bulbs which had been planted some weeks previously. The leaves grow most actively soon after they have burst through the

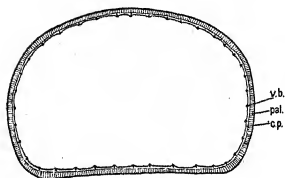


FIG. 2. Diagrammatic representation of cross section of middle region of leaf of onion. *pal.* pallid tissue; *c.p.* colourless parenchyma; *v.b.* vascular bundle. $\times 7$.

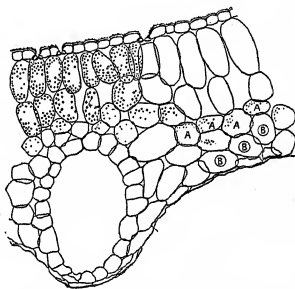


FIG. 3. Transverse section of middle region of onion leaf, drawn with Zeiss D.D. and 5.5 objective (Beck).

sheath which at first surrounds them. At this time the growth involves the entire length of the leaf, but after four or five days it is practically restricted to the basal region. This basal region continues to grow slowly for a week or more, according to the age of the plant. When all growth in length has ceased, a leaf generally falls over, making a sharp bend in the middle or near the base, and though such leaves may live for many days they were generally more or less flaccid and were avoided for experimental purposes.

The onion leaf is a hollow cylindrical structure for the greater part of its length, but the central cavity is filled with a delicate parenchymatous

tissue at the extreme tip and the base. The leaf has no stereome and is supported by the turgor of the thin-walled cells, more or less aided by the framework of slender vascular bundles. The epidermis is cuticularized and has particles of wax embedded in it which prevent the leaf from being easily wetted externally, even when kept under water for many hours. Immediately beneath it are two layers of typical palisade cells, arranged with their long axes perpendicular to the length of the leaf and occupying more than half the thickness of the tissue. Beneath these and at right angles to them is a single layer (or more in the region of the bundles) of shortly rectangular cells (A, A, Figs. 3, 4), containing fewer chloroplasts than the palisade cells, and within these are two or three rows of much more elongated cells (B, B, in Figs. 3, 4), the innermost of which are colourless

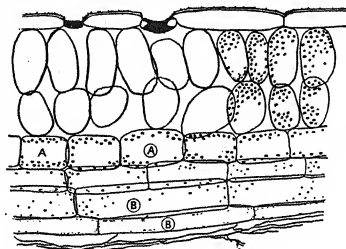


FIG. 4. Longitudinal section of middle region of onion leaf, taken between the bundles; drawn with Zeiss D.D. and 5.5 objective (Beck).

and contain only a watery plasma with a few degenerated plastids. On the interior of these cells are the collapsed and broken remains of the parenchymatous cells which once occupied the central cavity.

The mean isosmotic equivalent of the cell-sap was determined by applying the tissue-tension method of de Vries to the tissues previously well soaked in water, and was found to vary from 0.20 to 0.23 *gram. M. cane-sugar*, according to the age of the leaf, the older leaves giving the higher values. By repeated microscopic measurement it was found that at these strengths the only cells which show any sign of plasmolysis were the vertically elongated cells internal to the palisade (A and B in Fig. 4).

For this purpose a longitudinal section was cut from a turgid piece of leaf mounted in water, and an uninjured cell of the interior selected and measured by a micrometer scale and a high-power lens. The section was supported under the cover-glass by two strips of paper, and was irrigated with solution of any concentration by means of a narrow strip of linen dipping into a beaker and resting on the stage of the microscope.

Measurements were made at intervals, and when a contraction seemed complete a somewhat stronger solution was substituted. By this means it was found that the cells of the same section behaved differently in the same solution, and some light was also thrown on the behaviour of the tissues with hypertonic solutions.

In one experiment, in which the section was irrigated successively with solutions of 0.23, 0.25, and 0.30 grm. M. sugar, cells corresponding in position to B, B (Figs. 3 and 4) were observed, and each passed through the phases illustrated in Fig. 5.

Now the cells of which this cell is typical are just plasmolysed by the solution 0.23 grm. M. and have no longer therefore any turgor, yet on the addition of stronger solutions they undergo further shrinkage. Their walls

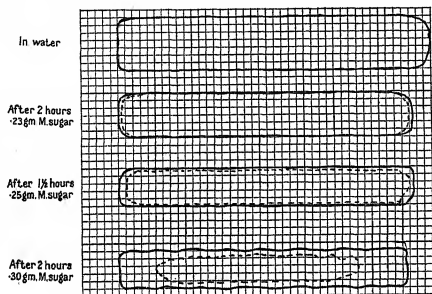


FIG. 5. A single cell from a longitudinal section of a turgid onion leaf, showing stages in plasmolysis with successive sugar solutions, drawn to scale. (1) In water. (2) After 2 hours 0.23 grm. M. sugar. (3) After 1½ hours 0.25 grm. M. sugar. (4) After 2 hours 0.30 grm. M. sugar.

first showed signs of crumpling after some hours in 0.3 grm. M. sugar, and with still stronger solutions the walls collapse altogether upon the shrunken protoplast. This complete collapse of the inner cells appears to be due in the first instance to the constraining effect of the palisade cells, which were all plasmolysed by the 0.3 grm. M. solution, but it also probably indicates an imperfect permeability of the cell-walls for sugar molecules. In any case, the cell-wall does not always remain completely extended after the protoplast is withdrawn from it, as is often assumed to occur in the plasmolysis of plant cells, but with hypertonic solutions other factors cause its further contraction. This leads to a prolonged shrinking of the whole tissue with hypertonic solutions, which is not primarily a question of plasmolysis, but is a sort of mechanical 'settling down' of the cellulose walls.

B. *Dandelion Scapes.*

It was found that a dandelion scape finishes its most active growth in length just before the opening of the inflorescence. In the cases observed, the flowers remained open for two days, and during this time there is slight

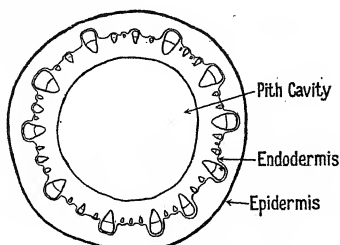


FIG. 6. Diagrammatic representation of transverse section of middle region of dandelion scape at flowering period. $\times 10$.

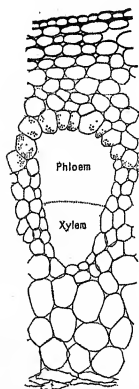


FIG. 7. Part of transverse section of middle region of dandelion scape taken between the larger bundles; drawn with Zeiss D. D. and 5.5 objective (Beck).

growth in length confined to the uppermost tapering part of the scape. After flowering, the bracts close over the inflorescence, and in about a fortnight they reopen for the dispersal of the fruits. During this fortnight there is active growth in length in the *basal* region of the scape, and a certain amount of increase in diameter is also attained—by tangential stretching rather than by meristematic ac-

tivity. In collecting material, lengths were always cut from the basal region of a straight healthy scape, bearing unfaded flowers. They were transferred to a beaker of water on the spot, and were supported vertically to avoid geotropic effects during the interval between collecting the material and making an experiment.

The scape has a very simple structure and, excepting for the epidermis and the ring of vascular bundles, is entirely made up of parenchymatous cells, with numerous intercellular spaces between them. The epidermal cells are thinly cuticularized. Two or sometimes three layers of hypodermal cells are narrow radially, elongated vertically, and thickened with cellulose on their tangential walls. The remaining cortical cells are thin-walled and rather longer than broad; the pith cells are rectangular with square ends and at least twice as long as broad. The pith cavity is clearly formed by the rupture of the central cells at an early stage, and the remains of their torn walls adhere to the inner living pith cells. The outer cortical cells contain numerous scattered chloroplasts, but starch

was never found in any abundance except in the endodermis. In an old scape bearing the nearly ripened fruits, no starch at all could be detected except in the endodermis in the uppermost region of the scape. There is a ring of vascular bundles embedded in the ground tissue, and laticiferous elements occur in the pericycle, but no interfascicular cambium develops, and for the purposes of this research these tissues can be disregarded.

Thin strips of turgid dandelion scapes show well-marked tissue tensions. By the method of de Vries the mean isosmotic strength of the cell-sap was found to be equivalent to a solution of 0.42 grm. M. cane-sugar at the beginning of the flowering season (April in 1915), and 0.53 at the end (late June). This was confirmed by microscopic observation, but no exact measurement of single cells was made as for the onion.

SECTION V. CHOICE OF A SOLUTION FOR INVESTIGATING RATE OF PLASMOLYTIC SHRINKAGE.

For the plasmolysing solution it is desirable to use one which neither injures nor penetrates the protoplast. The solution which has most frequently been used is cane-sugar, which fulfils both these conditions more or less satisfactorily, and can be obtained readily in a pure form.

The choice of a suitable concentration is a matter of more difficulty. The simplest course seemed to be to find the isosmotic equivalent of the cell-sap and to use a solution which was just hypertonic to this. By examining thin strips of dandelion scapes and onion leaves after the manner of de Vries, it was found that a sugar solution of 0.5 grm. M. was just hypertonic to the cell-sap of the dandelion, and 0.3 grm. M. was hypertonic to that of the onion. These solutions were first used for the various temperatures, but owing to the difficulty of deciding when the contractions had ended, and to the variable forms of the curves obtained, both stronger and weaker solutions were subsequently experimented with. The solutions thus investigated were respectively 0.731,¹ 0.5, 0.3, and 0.18 grm. M., all being made up to 'weight-normal' standards. The whole contraction produced by these solutions was measured by a microscope on a micrometer screw travelling horizontally, as well as by the optical lever apparatus, and was found to vary somewhat with the age of the material, but approximate values are given in Table I.

TABLE I.

Sugar solution.	Dandelion.		Onion.	
	Contraction (scale divisions).	% Contraction of original length.	Contraction (scale divisions).	% Contraction of original length.
0.18 grm. M.	—	—	20-50	0.3-0.5 %
0.30 grm. M.	15-40	0.2-0.4 %	200-350	3 %
0.731 grm. M.	200-250	2-2.5 %		

¹ This strong solution (25 %) was largely used by van Rysselberghe.



It will be seen that there is a much greater shrinkage with strong than with weak solutions, but that the contraction is easily measurable with the weakest solution employed.

In dealing with plasmolysis rates at different temperatures, it is necessary to have some definite standard of comparison. The length of the pieces used varied from 28 to 30 mm.—being usually nearer 29 mm.; the absolute contraction, therefore, was necessarily also variable. It seemed best to relate the total linear contraction observed in each experiment, therefore, to a fixed length which was taken as 100, the observed contractions at any point being thus expressed as percentages of the total contraction. The ordinates in Figs. 11, 13, 14, are of this type.

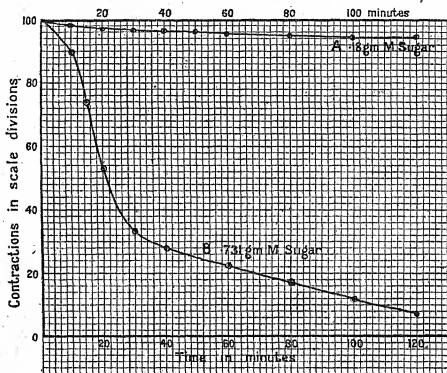


FIG. 8. Curves comparing effect of 0.18 and 0.731 gm. M. concentration of sugar on the plasmolytic contraction of onion leaves, plotted to same scale. Ordinates are divisions of scale.

For this method it was necessary to know when the plasmolytic contraction in any solution had ceased, a thing almost impossible to judge in the case of strong solutions which gave a continuous slow contraction towards the end, for many hours. In Fig. 8 the actual course of contraction is shown of dandelion scapes under identical conditions at 14° C. but at different concentrations of sugar; curve B shows part of this slow final shrinkage, and curve A on the same scale the slight but definitely ending contraction with a subtonic solution. In addition to this difficulty, with strong solutions there is often a slower rate of plasmolysis at the beginning of an experiment than after the first half-hour. This can only be due to some secondary factor disturbing the normal course of contrac-

tion, for the difference in concentration between the cell-sap and the outer solution is then at its greatest and, other things being equal, must give the fastest rate of plasmolysis in the first phase of an experiment. For these reasons it is hardly possible to estimate closely the effect of temperature on the course of plasmolysis produced by means of markedly hypertonic solutions, and in all the critical experiments on temperature effects in this research subtonic solutions alone were employed.

SECTION VI. RATE OF PLASMOLYTIC SHRINKAGE IN A SUBTÖNIC SOLUTION.

It was necessary to make trial experiments in order to ascertain which strength of subtonic solution would be most suitable for the experiments at different temperatures. The general considerations were that the solution should be weak enough to give a definite end-point in not more than two or three hours at ordinary temperatures, and not too weak to give a contraction of less than about 20 divisions on the scale. In practice, it was found that solutions which gave contractions varying from 30 to 50 divisions on the scale gave a definite ending in about two hours at ordinary temperatures, and also gave a curve of nearly logarithmic form. It can be shown, on theoretical grounds, that this is the form of curve which would be expected with dilute solutions apart from any secondary disturbing causes. It was found that the best results were given with solutions of 0.18 grm. M. sugar for the onion and 0.3 grm. M. for the dandelion; these two cases will be considered separately.

A. Leaf of Onion.

The mean osmotic pressure of the cell-sap of onion leaves was found to vary with the age of the leaves, those which had only just finished their growth being isotonic with a sugar solution of 0.20 or 0.21 grm. M., and older ones with a solution of 0.23 grm. M. (i.e. four to five atmospheres pressure). The original solutions tried were 0.25, 0.20, 0.18, and 0.15 grm. M., but the last was at once rejected on account of the small contractions given with some leaves.

Even with great care in the choice of material and in repeating the same experimental conditions, there was a certain amount of variation in the rate of plasmolysis at the same temperature. At medium temperatures, therefore, it was usual to perform several experiments under the same conditions, and to select the ones which gave the curve of approximately logarithmic form (e.g. A, in Fig. 13).

It was found that at temperatures above that of the laboratory there was a short period of expansion of the tissues when first the material was fixed in place. At temperatures not higher than 30° C. this was

followed by a long period of constant length. In experiments with sugar, therefore, at these temperatures the solution was not applied until the initial expansion had ended.

At temperatures above 30°C . the preliminary expansion is maintained for a time, but is soon followed by a considerable contraction, probably owing to an escape of cell-sap caused by the increased permeability for salts at high temperatures. This contraction appeared in about two hours in an experiment at 36°C . (Fig. 9, A), and since the plasmolytic shrinkage is completed within half an hour at that temperature, no correction for the temperature effect is needed.

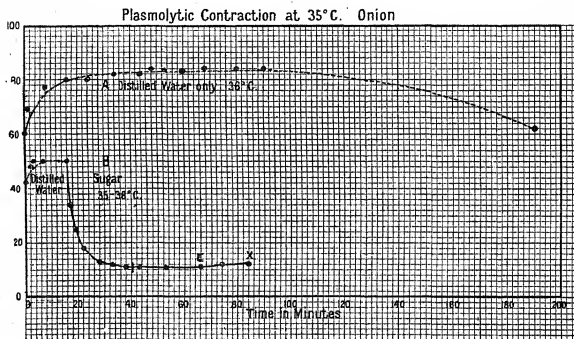


FIG. 9. Curves showing effect of temperature on onion in distilled water at 36°C . (A) and plasmolytic contraction at the same temperature, (B) with 0.18 gm. M. sugar. Ordinates are observed scale readings.

The contraction at that temperature (Fig. 9, B) was followed by an expansion indicated at E, X (Fig. 9, B), which is almost certainly due to the continued entry of the sugar molecules into the cells, but the expansion was not followed to its conclusion.

At temperatures above 36°C . there was a marked contraction appearing after half an hour or even less of exposure to the water-current (Fig. 10, A). It therefore seemed advisable to apply the solution as soon as the initial expansion had ended, to avoid any unnecessary temperature effect. The plasmolytic contraction was finished in ten minutes or less, and the temperature contraction was then again evident (Fig. 10, B, C, D). In all the curves at these high temperatures there appeared to be an antagonism between the tendency to expand with entry of sugar, and to contract as the effect of the prolonged high temperature. An analysis of these secondary

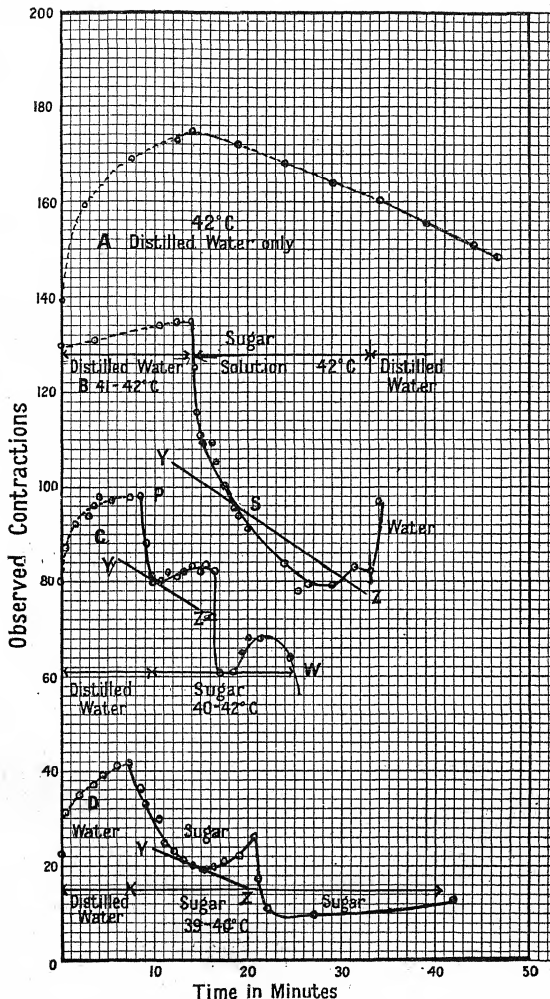


FIG. 10. Curves showing effect of temperature on onion in distilled water (A), and effect of subtonic sugar solution (0.18 gram. M.) (B, C, D). Ordinates are readings on scale.

effects has not been made, but by using the first period of contraction immediately after the application of the sugar current, and by correcting for the concurrent temperature effect curves, of plasmolytic shrinkage from 37° to 42° C. were obtained which were of similar form to those at lower temperatures, but correspondingly steeper. The method of correcting the curves is shown in Fig. 10, where the oblique line *Y, Z* is taken as the base line of the curve. This is, in effect, to subtract from the observed con-

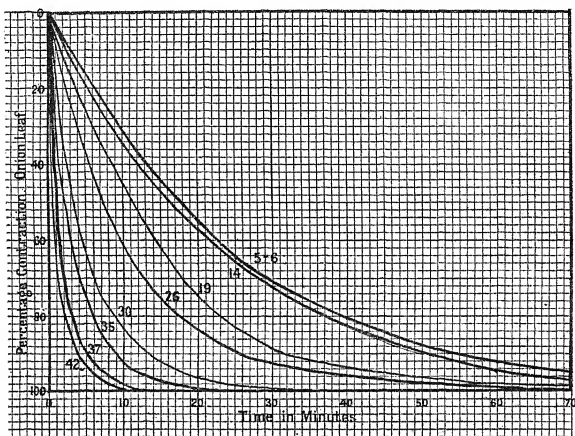


FIG. 11. Chart of the course of the shrinkage-time curves of onion leaf at different temperatures. All the shrinkages were carried out in subtonic sugar solution (0.18 grm. M. cane-sugar). The individual curves are representative ones from a group taken at each temperature, and in all cases the absolute shrinkage is brought to a standard amount of 100 units as represented by the ordinates.

traction at any point the temperature effect, estimated from the curve *A* previously obtained with distilled water only.

It was not easy to repeat exactly the conditions of a high temperature experiment, but close temperature intervals of successive experiments were selected in order to make the results as representative as possible.

After thus selecting a suitable curve for each of a number of temperatures, these were all plotted to the same scale, the contractions at any point being expressed as percentages of the total contraction in that experiment. These temperature curves for the onion are reproduced in Fig. 11, and the actual contractions observed are given in Table II.

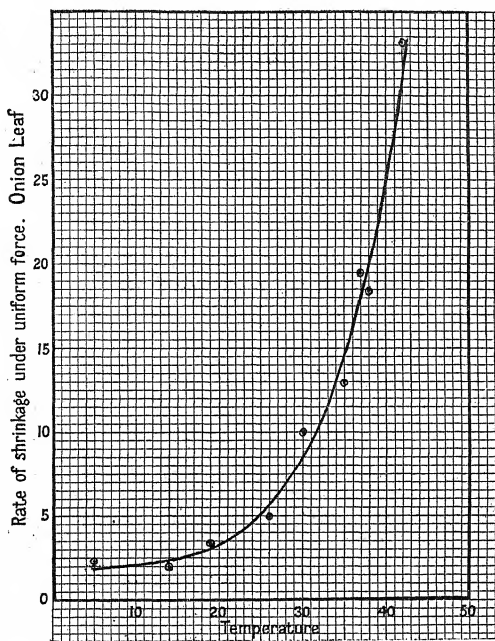


FIG. 12. Curve showing the rates of shrinkage of tissue of onion leaf under uniform external osmotic compression but at different temperatures. This curve exhibits the alteration of protoplasmic permeability brought about by temperature. The actual ordinates are the values of the tangents of the shrinkage curves where the 50 % shrinkage line cuts them in series.

TABLE II.

Temperature.	Observed contraction in scale divisions.	Time taken to complete contraction. Min.
5° C.	19	95
14° C.	20.5	85
19° C.	41	100
26° C.	42.5	100
30° C.	34	35
35° C.	36.5	25
37° C.	20.8	12
38° C.	33	10
42° C.	28	10

The tangents of these curves at any stage in the contraction gives a measure of the rate of the contraction at that stage, and these values found for different curves at the same stage and plotted to the corresponding temperatures as abscissae give the relative rate of plasmolysis. The values of these tangents for the onion are given in Table III.

TABLE III.

Rate of plasmolysis at 30 %, 50 %, and 70 % contraction, in onion, irrigated with 0.18 grm. M. sugar.

Temperature.	Amount of contraction completed.		
	30 %.	50 %.	70 %.
5° C.	2.8	2.3	0.77
14° C.	3.4	2.0	1.4
19° C.	4.5	3.4	2.5
26° C.	6.2	5.0	2.7
30° C.	14.3	10.0	5.0
35° C.	26.6	12.9	8.5
37° C.	25.4	19.5	18.3
38° C.	23	18.4	11.9
42° C.	51.8	36.2	12.1

From this table it can be seen that at all stages of the plasmolytic shrinkage produced by these subtonic solutions, there is a considerable increase of the rate of shrinkage with rise of temperature. The temperature effect seems to be much more marked towards the end of the contraction, but as the endings of experiments were always more liable to possible errors of interpretation than any other part it seems better to take the values at mid-plasmolysis, and these are plotted to the corresponding temperatures in Fig. 12. The coefficients of increase of permeability deduced from the curve are:

Temperature range.	Coefficient of increase for 10° C.
5°-15°	1.4
10°-20°	1.5
15°-25°	2.0
20°-30°	2.6
25°-35°	2.9
30°-40°	3.0

B. Scape of Dandelion.

In order to confirm these results, similar experiments were carried out with the scapes of dandelions, which have a more homogeneous structure than onion leaves. The solution employed was 0.3 grm. M., and was found by trial as for the onion. It was afterwards found that the dilute solutions used for each plant bore the same ratio to the corresponding isotonic solution (1:1.4), which is in itself a somewhat striking testimony to the trustworthiness of the apparently arbitrary method of selection of these subtonic solutions.

The osmotic pressure of the cell-sap of dandelion scapes was found to vary with the advance of the flowering season, being isotonic with a solution of 0.42 grm. M. at the beginning (i. e. early April in 1915), and with a solution of 0.53 grm. M. at the end (i. e. late June): this represents a varying pressure of about 9 to 11 atmospheres. During the final experiments on this material, which were made in the latter part of June, the contractions given by the 0.3 grm. M. solution were reduced to only 8 or 10 divisions, and in order to obtain one of the high temperature results it was necessary to increase the strength of the sugar to 0.40 grm. M., when a contraction of 23 divisions was obtained at a temperature of 42° C.

Dandelion Plasmolytic Contraction % at 19° C.

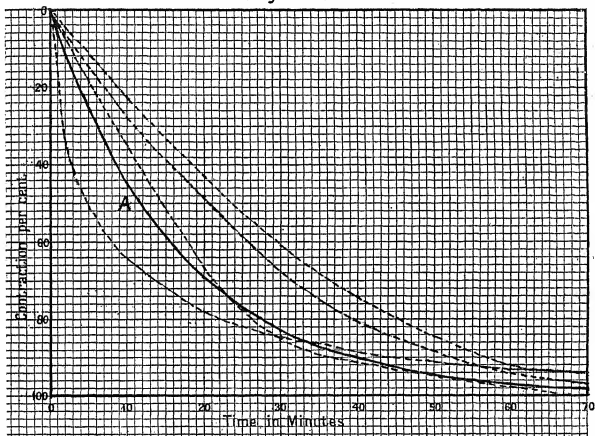


FIG. 13. Curves showing percentage contraction of dandelions at 19° C. with subtonic solution (0.3 grm. M. sugar). Ordinates are percentages of total contraction.

As before, a number of experiments were made at each temperature, and the most typical curve selected to represent each temperature. As a rule the separate individual curves fell very close together when plotted to ordinates of percentage contraction. In Fig. 13 is illustrated the greatest divergence found for any one temperature. In this set are however included curves from material previously long irrigated with distilled water as well as those for short soaking in tap-water. From such a set, the curve A from material soaked two hours in tap-water was selected as the one falling with the simplest logarithmic regularity throughout.

In experiments with dandelion scales it was often noticed that after all plasmolytic contraction had ceased a gradual re-expansion of the tissues occurred, while the tissues were still surrounded with the same sugar solution. This naturally suggested a slow entry of the sugar molecules into the cells, causing an extension of the protoplast again. In order to test this hypothesis, the water-current was turned on again, and in the recovery of the tissues which ensued the extension was always slightly greater than the initial contraction had been. Since every care had been taken to ensure an initial turgidity of the tissues, the only explanation appears to be that

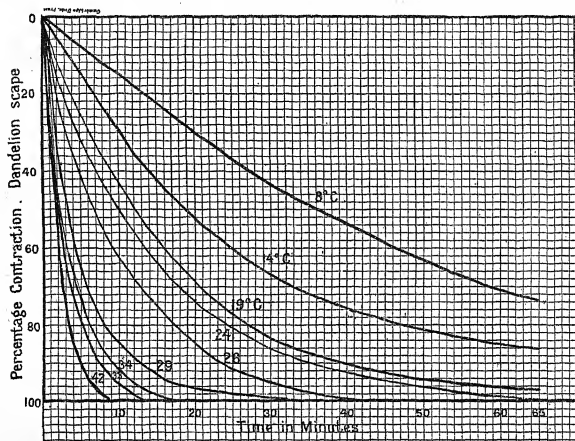


FIG. 14. Chart of shrinkage-time curves of dandelion scape at different temperatures. Interpretation as in Fig. 11.

there had been a penetration of the sugar molecules, which had therefore raised the osmotic pressure of the cells, and enabled them to take up more water at the same temperature than they could formerly have done. This point seems worth emphasizing, since sugar has always been regarded as the most impenetrable of substances with regard to the protoplast.

The experiments at high temperatures are attended with the same difficulties as in the onion, and the curves obtained at temperatures above 35°C. were corrected in the same way for the temperature effect. The collective curves for all the temperatures investigated are shown in Fig. 14, and the corresponding contractions and times are given in Table IV. It can

be seen that the general distribution of the curves with respect to temperature is very similar to that for the onion excepting at low temperatures.

TABLE IV.

Table of contractions at different temperatures with 0.3 grm. M. sugar.

Temperature.	Contraction as divisions on scale.	Time.
8° C.	36.5	3 hr. 8 min.
14° C.	34	4 hr. 45 min.
19° C.	36	1 hr. 27 min.
24° C.	39	1 hr. 5 min.
26° C.	15	45 min.
29° C.	26.5	35 "
32° C. { (a)	32	28 "
{ (b)	25.8	21 "
34° C.	29	18 "
33° C.	8.6	16 "
36° C.	24.2	14 "
42° C. { (a)	22.5	10 "
{ (b)	27	14 "

The tangents of these curves at different stages in plasmolytic contraction are given in Table V, and the values at mid-plasmolysis plotted as before to the corresponding temperatures give the curve reproduced in Fig. 15.

TABLE V.

Temperature.	Rates of plasmolytic contraction at		
	30 %	50 %	70 % of total contraction.
8° C.	1.4	1.0	0.79
14° C.	2.8	1.7	0.96
19° C.	4.3	2.7	1.9
24° C.	5.1	3.6	1.9
26° C.	7.2	4.5	2.4
29° C.	16.7	9.3	3.5
32° C. { (a)	18.5	10.3	3.9
{ (b)	16.6	12.2	5.1
33° C.	23	13.6	9.1
34° C.	15.1	14.9	6.7
36° C.	28.9	15.6	8.9
42° C. { (a)	35.9	22.8	15.5
{ (b)	31.2	23.7	17.7

From this curve for 50 per cent. contraction, the following temperature coefficients may be derived:

Temperature range.	Coefficients of increase for 10° C.
5°-15°	—
10°-20°	2.3
15°-25°	3.3
20°-30°	3.8
25°-35°	3.0
30°-40°	2.6

COMPARISON OF THE TWO PLANTS.

Although the two types of material selected are not physiologically or morphologically very similar, their general behaviour in relation to temperature is obviously in agreement. In Figs. 12 and 15 the two curves have the same general relations. On closer comparison it will be seen that

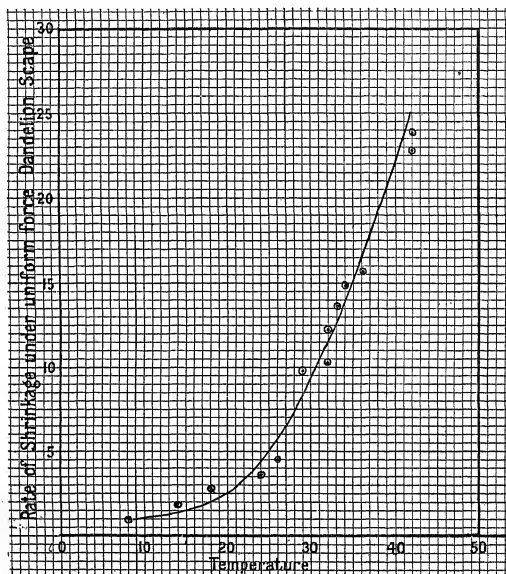


FIG. 15. Curve of relation to temperature of the rate of shrinkage of tissue of dandelion scape. Interpretation as in Fig. 12 (p. 299).

for temperatures of 25°C. and upwards the values of the relative permeability are practically identical, while below 25° the dandelion values decline faster than those of the onion. It cannot yet be said whether this is related to the fact that the dandelion is a summer plant, the experiments being done in April to June, while the onion leaves had lived through the winter and were investigated from January to March.

The agreement and divergence between the two plants is best brought

out by taking a common relative value of unity for their permeability at the middle point 25° C. We then have the appended series of values :

	5°.	10°.	15°.	20°.	25°.	30°.	35°.	40°.
Onion	0.36	0.44	0.50	0.66	1.0	1.7	2.9	5.0
Dandelion	—	0.22	0.30	0.50	1.0	1.9	3.0	5.0

SECTION VII. CRITICAL CONSIDERATION OF THE RELATION BETWEEN PERMEABILITY AND TEMPERATURE PUT FORWARD BY F. VAN RYSSSELBERGHE.

The only other known published results on the exact relation between permeability of protoplasm to water and temperature are those brought forward by F. van Rysselberghe¹ in 1901. As these results differ completely as to the effect of temperatures above 20° C. they must now be carefully examined. Studies were made of the time taken to plasmolyse and to deplasmolyse different kinds of material, such as cylinders of elder pith, epidermal cells of *Tradescantia*, and *Spirogyra*. Each kind was examined at the temperatures set out below, and harmonious ratios for the relative rates of the observed processes at different temperatures were obtained. The ratios are appended, the rate at 0° C. being taken as unity (van Rysselberghe, p. 189) :

Temperature	0° C.	6° C.	12° C.	16° C.	20° C.	25° C.	30° C.
Rate	1	1.9	4.5	6.25	7.15	7.5	8

It will be seen that above 20° C. there is practically no further increase in permeability, and the graphic record Fig. 16 copied from van Rysselberghe's paper shows how this curve agrees broadly with our present results up to 20°, but differs completely in type afterwards.

Many of his types of measurement must have been difficult to carry out with precision; such as the exact time for complete plasmolysis of a cell, and still more so the exact time taken by such a cell to recover its normal state. The data provided for this type of observation do not lend themselves to critical analysis, but the measurements of the shortening of pith are recorded at several successive points of time, and it is proposed to consider them carefully. Long cylinders of elder pith were plasmolysed in strong sugar solution. The procedure was as follows: the cylinders were freshly cut from the plant, freed from traces of wood, and soaked for six hours in water at 16° C., during which time they expanded from 100 mm. to 114 mm. The cylinders were then cut longitudinally into two half-cylinders, as equal as possible. One half was placed in sugar solution at 0° C. as a control, and the other kept in sugar solution at one of the temperatures specified above. The cane-sugar solution used for plasmolysis was 25 per cent. (0.731 grm. M.). At all temperatures the half-cylinders shrank

¹ F. van Rysselberghe: Influence de la température sur la perméabilité du protoplasme vivant pour l'eau et les substances dissoutes. Bull. Acad. Roy. de Belgique, 1901, pp. 175-221.

a great deal in length, ultimately 37 per cent., but did not alter appreciably in diameter. At intervals of two hours the lengths of the half-cylinders were measured, and the amount of shrinkage in mm. is recorded (*loc. cit.*, p. 184). In Fig. 17 the data are presented in graphic form, the dots being the original data and the broken lines joining them being an attempt to reconstruct the course of the curves over the gaps in the record. In this form the results can be easily compared with the curves set out in the body of this paper.

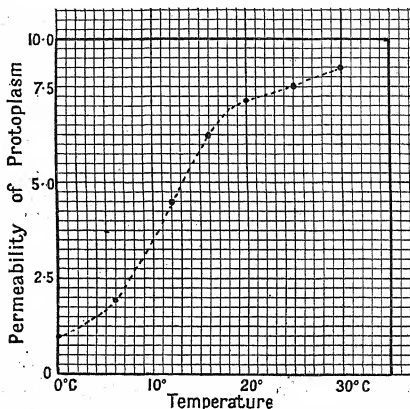


FIG. 16. Relation of temperature and permeability of protoplasm. From F. van Rysselberghe (*loc. cit.*, p. 190).

It will be seen that, as is to be expected, each curve begins with a more rapid fall, and the rate of plasmolysis gradually declines to zero when the shrinkage is complete at the pith-length of 72 mm. For the low temperatures the ends of the curves are beyond the twenty-four hours at which the records cease, while for the high temperatures the contraction is practically complete when the first record is taken at two hours. The first matter for criticism is van Rysselberghe's method of extracting a temperature-relation from these data. He takes what may be called a *vertical* section of the chart; that is to say, the amount of contraction shown by the respective curves at two hours of time is adopted, the ratios being those set out on p. 305. Now it should be clear that the only vertical comparison of the curves which has any real significance is the impossible one that should be taken an instant of time only after the beginning. As each curve begins with the same big osmotic pressure force (the difference of concentration of

the cell-sap and of the bath of cane-sugar), at that time the ratios of the amounts of contraction would be significant, but as these initially equal forces die away quickly where the curve is steep, and slowly where it slopes gently, they henceforth have all different forces at any point of time, and the effect of such vertical comparisons becomes more and more remote from any significance as time proceeds. It is obvious that at twenty-four hours the vertical comparison would indicate the same contraction for all temperatures of 12° C. and upwards.

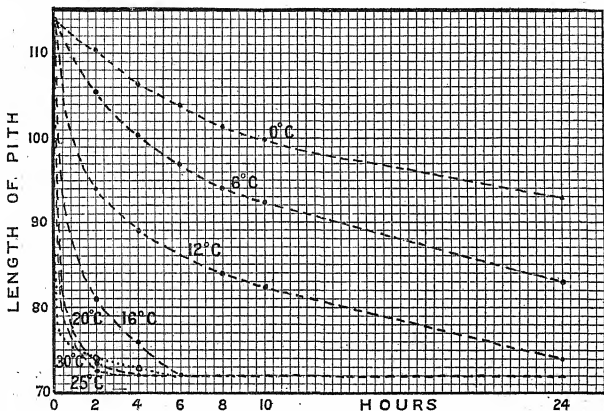


FIG. 17. Course of plasmolysis of elder pith in 0.731 grm. M. saccharose solution at different temperatures: the data taken from F. van Rysselberghe's table. The ordinates are actual lengths in mm.

To extract the real temperature relation from such a set of curves a *horizontal* section must be made through the curves, and comparison thus made of relative rates, because where any one horizontal line cuts each curve there the force at work is equal throughout the set.

Tangents measured at these points will give a true index of the effects of the respective temperatures. Such treatment must give a different picture of the temperature effect from that presented by van Rysselberghe.

The data on the time-relations of deplasmolysis are curiously imperfect and difficult to analyse. The conclusions drawn from them suffer from the same defect as those on plasmolysis. It becomes therefore clear that van Rysselberghe's conclusions lack any precise experimental foundation.

There is a further disturbing matter that shows itself very clearly when these data are presented in a graphic form. It is thereby made obvious that

whereas the curves from 0° to 16° C. are well separated by temperature effect, the curves for 20°, 25°, and 30° C. are practically identical. This van Rysselberghe regards as a true index of the temperature effect. While this physiological view is entirely different from that substantiated in this paper, it is interesting that an appearance of the same result can be got for dandelion scapes by our method, *but only by using very strong plasmolysing solutions*. The interpretation of this phenomenon seems to be that there is an absolute upper limit to the rate at which the mechanical tissue-system of cell-walls can collapse and shrink. If so, then however fast and suddenly the protoplasts are crushed by the strong osmotic pressure of 25 per cent. cane-sugar, the cell-wall reticulum—which is what is measured—cannot follow at a rate above a certain limit. In van Rysselberghe's experiments that limit seems to be reached with 0.731 grm. M. cane-sugar at 20° C. A great part of each contraction curve must consist of the 'settling down' phase, and over this permeability of protoplasm has no control.

The moral to be drawn from these considerations is that, for demonstrating the full accelerating effect of temperature, it is essential to employ plasmolysing solutions which are *subtonic*, so that the force exerted on the cell is slight. At low temperatures the absolute rate of contraction will be very slow, but it will then be possible to demonstrate the continued accelerating effect of high temperatures without the records being cut off by some mechanical limiting factor which has nothing to do with permeability of protoplasm.

SECTION VIII. SUMMARY AND CONCLUSIONS.

The method of following the rate of shrinkage of strips of tissue bathed in solutions that tend to plasmolyse them has been developed as a means of measuring the permeability of protoplasm to water.

Hollow leaves of onion and hollow scapes of dandelion have been selected for investigation because they present on their inside a natural surface of uncuticularized thin-walled cells.

Apparatus has been employed by which a current of liquid flows continuously through a short cylinder (30 mm.) of the material under investigation. By suitable arrangements a solution of sugar can be instantaneously substituted for the water-current and vice versa without any alteration of temperature.

An 'optical lever' is connected to the upper end of the tissue cylinder and alterations in length of 0.003 mm. (one part in ten thousand) are made evident.

The change of protoplasmic permeability with temperature between 5° C. and 42° C. has been carefully studied by this technique.

Such experiments cannot be satisfactorily carried out with strong plasmolysing solutions, as the shrinkage is then very quick at even moderate

temperatures, and there seem to be mechanical limitations to a quicker shrinkage being manifested at higher temperatures. With the weak subtonic solutions adopted, 0.18 grm. M. for onion and 0.3 grm. M. for dandelion, plasmolysis is not reached, contraction is comparatively slow, and the experimentation is confined to the strictly normal state of falling turgor, without actual separation of the protoplast from the cell-wall.

A large number of records with such subtonic sugar solutions have been made at different temperatures. The agreement between individual records at the same temperature is generally good, though the absolute shrinkage dealt with is only 0.12 mm. Fig. 13 illustrates the most extreme divergence observed at any one temperature. Sufficient records have been taken to establish that the most regular curves follow a nearly logarithmic course.

The most regular medium curve of each temperature group has been selected as representative, and these are charted together—onion, Fig. 11, dandelion, Fig. 14—all the contractions being brought to the uniform scale of 100 units.

These curves show a continuously increasing rapidity of shrinkage with rise of temperature over the whole scale.

Up to 35°C. it is found that, when perfused with water alone, the tissue length remains constant for a long period of time. Above 35°C. some shrinkage takes place in water alone, so that the further shrinkage when perfused with sugar is not quite a simple effect. The curves charted for these high temperatures represent sugar-shrinkage less the amount of water-shrinkage during the corresponding time. Such a corrected curve errs, if at all, on the side of being not steep enough.

To obtain the true measure of temperature-effect from these curves in the charts, it must be remembered that the rate of shrinkage, as expressed by the slope of the curve at any point, is a product of two factors. Of these, one is the compressing force acting on the protoplast and causing exudation of water (the osmotic force of the perfused solution, plus the elastic pressure of the cell-wall, less the osmotic pressure of the cell-sap); and the other, the permeability of the protoplast for water at the temperature prevailing.

The influence of the permeability factor can only be obtained from a set of temperature shrinkage-curves by comparing them at really corresponding points, that is at points where the compressing force is equal throughout the set. Such corresponding points are those lying on any one horizontal line on the chart, parallel to the abscissa-axis.

For the three lines corresponding to 30 per cent., 50 per cent., and 70 per cent. of the total contraction, respectively, the exact slope of every curve has been ascertained by measuring the values of the tangents at these points on a large-scale diagram. The values of these tangents, representing the relative rates of contraction, are set out for the three series

on pp. 300, 303, and the most acceptable series for the two plants (the 50 per cent. series) are plotted against temperature in Figs. 12 and 15.

As the force at work was, by selection, equal in all these cases, the differences of rate shown are true measures of the state of the permeability. It is evident that the augmenting effect of temperature increases to the highest temperature investigated.

These curves are in opposition to the generally accepted curve for this relation put forward by F. van Rysselberghe in 1901 (see Fig. 16, p. 306), which indicates hardly any rise of permeability after 20° C. In Section VII van Rysselberghe's data are critically examined, and it is pointed out that his method of deriving a temperature-relation from his data (plasmolysis of cylinders of pith in 25 per cent. (0.731 grm. M.) cane-sugar) cannot give a true relation.

The data themselves are also open to some criticism on account of the very strong plasmolysing solution used. Here the effective force at work is so great that the shrinkage is very quick even at medium temperatures, and there seem to be mechanical limitations to any quicker shrinkage at high temperatures even though the protoplasm becomes more permeable.

Further consideration will be devoted to the theoretical significance of the experimental results now brought forward in a later paper of this series. It will suffice at present to give the quantitative conclusions as follows:

1. The permeability of protoplasm for water, as measured by the rate of tissue-shrinkage in a dilute sugar solution, is increased continuously by temperature up to the highest temperature investigated—42° C.

2. Taking the value of the permeability at 25° as unity, the following relative values are established:

Temperature	5°	10°	15°	20°	25°	30°	35°	40°
Onion	0.36	0.44	0.50	0.66	1.0	1.7	2.9	5.0
Dandelion	—	0.22	0.30	0.50	1.0	1.9	3.0	5.0

3. An approximate measure of this increase is given by the following sets of temperature-coefficients for increase of permeability with rise of 10° C.:

Temperature.	Onion leaf.	Dandelion scape.
5°-15°	1.4	—
10°-20°	1.5	2.3
15°-25°	2.0	3.3
20°-30°	2.6	3.8
25°-35°	2.9	3.0
30°-40°	3.0	2.6

A Note on the Vegetative Anatomy of *Pherosphaera* Fitzgeraldi, F. v. M.

BY

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With one Figure in the Text.

PHEROSPHEERA is a coniferous genus separated by Archer from *Dacrydium*, but by some botanists placed within this genus. It includes only two species, both Australian—*P. hookeriana*, Archer, restricted to alpine Tasmania, and *P. Fitzgeraldi*, F. v. M., only known to occur on the Blue Mountains of New South Wales.

The latter species is a dense, prostrate little shrub, whose slender twigs are clothed with numerous narrow, keeled, leaves, which are about 3 mm. in length. In their book 'The Pines of Australia', Baker and Smith state that the plant is 'found at the base of most of the chief falls', and add that their material (used in the present investigation) was obtained at the Lower Falls, Leura, in which site the shrub 'only grows where it can catch the drips from the falls'.

The remarkable nature of the habitat, recalling that of certain Hymenophyllaceae in tropical forests, caused me to examine the structure of the wood and leaves, in the hope of discovering a conifer truly hygrophytic in structure.

a. THE WOOD.

In its construction the wood of the stem by no means suggests a hygrophyte. In the thickest stem available the diameter was 4.5 mm., yet the number of annual rings present was thirteen. The radial thickness of each annual ring was therefore 0.173 mm., and thus represented 144 annual rings to the inch radius. The thickness of the annual rings increased outwards from the first to the tenth, but decreased in the remaining three rings; it is therefore probable that the stem had passed its maximum rate of growth in radial thickness.

All the tracheides of the secondary wood are thick-walled, and recall those forming the summer-wood (autumn-wood) of such a conifer as *Pinus*

sylvestris, especially as their lumina are narrow. But the inmost tracheides, often exactly one series, of the annual ring show wider cavities than elsewhere, and thus represent a microscopically thin 'pore-zone' of spring-wood.

The bordered pits of the tracheides are mainly on the radial walls, where they are by no means abundant, being uniseriate even in the spring-wood; their fusiform apertures are spirally directed, and in the summer-wood exceed in length the diameter of the chamber. Bordered pits also occur on the tangential walls, more especially of tracheides forming the outer boundary of the annual ring.

The medullary rays are all uniseriate and remarkable for their shallowness, being usually only 1 to 4, occasionally 5, cells in height. They consist solely of thin-walled parenchyma, in the lateral radial walls of which the pits are nearly equal to the height of the cell, or each pit of such a kind is replaced by two superposed ones.

THE LEAF.

The leaf is xerophytic in structure. In the first place the epidermis has a thick cuticle and otherwise thick walls, while the stomata are sunken. Secondly, there is a single layer of thick-walled hypoderma, which is continuous except within the stomatic apertures.

Contrasting with this tegumentary system is the very loose green tissue lying within and simulating palisade-parenchyma though excavated by a large intercellular system.

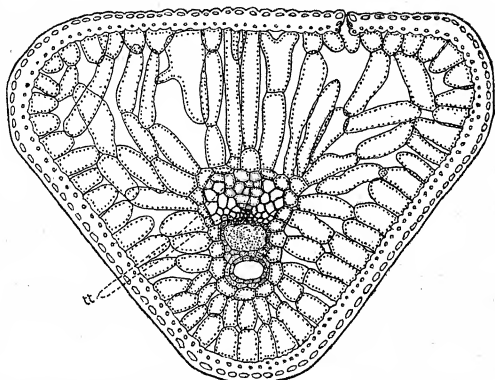
The centre of the leaf is occupied by a strand that is denser in structure, except that it includes a large median ventral resin-duct. The feature worthy of special note is the extensive development of the transfusion-tissue in comparison with the puny xylem proper.

The stomata are restricted to the upper face of the leaf, where they are ranged in several longitudinal rows. In each row the successive stomata are mostly separated merely by a single short epidermal cell, but nearer the ends of such a row these single short cells become replaced by longer ones, or by only two or several cells. The longitudinal rows of stomata are separated laterally from one another by only two lines of epidermal cells.

The communication of the stoma with the internal atmosphere of the leaf is sharply limited. For within each stoma the gap not only in the thick-walled hypoderma, but also in the mesophyll lying within, is very small; indeed the interruption in the latter tissue is limited to the local separation of either two or three cells, which, in surface section, bound an intercellular space whose oval outline approximately coincides with the periphery of the guard-cells.

The hypoderma at certain isolated spots, in transverse section, is as much as three cells in thickness.

The green tissue immediately within the hypoderma deviates from typical palisade-parenchyma as regards both shape and orientation of the cells and dimensions of the intercellular spaces. The general design of this loose tissue is that of two layers of elongated cells, simulating palisade-cells, and stretching between the hypoderma and the central strand of the leaf. These cells, and particularly those forming the outer layer, are mainly perpendicular to the leaf-surface, but those radiating from the central strand towards the lateral angles (in transverse section) deviate from this design so greatly that in the angles they are actually tangential to the local curved surface. The cells forming the outer green layer frequently are lobed either



Transverse section of leaf of *Pherosphaera Fitzgeraldi* (slightly diagrammatic).

laterally or basally, or have dilated bases; this is especially the case within the upper face of the leaf, so that here in transverse section a number of structures may be seen that apparently are short cells but really are the basal portions of long cells. The green cells of the outer series attain their maximum length towards the upper face of the leaf.

The cells forming the inner layer of palisade-like tissue on reaching the central strand may bend so that their inner portions run parallel to the length of the leaf and thus contribute to the outermost layer of the central strand: such cells consequently lose their palisade-like form, and in transverse section are liable to be mistaken for tissue confined to the central strand.

The cells forming the outermost layer of the central strand contain chlorophyll, are elongated in the direction of the leaf-axis, and may emit

lateral lobes that serve to connect them with the surrounding tissue. Thus there is no absolute distinction between the cells of this layer and of the inner palisade-like layer.

The remainder of the central strand is composed of :

- (1) parenchyma ;
- (2) the single median ventral resin-duct, showing a double epithelium ;
- (3) phloem ;
- (4) xylem, with transfusion tissue.

It was impossible to observe the minute details in the histology on the phloem in the dried herbarium material alone available.

The transfusion-tissue (*tt.* in the illustration) in transverse section extends from the feeble xylem in the form of two wings, which ascend towards the upper face and sometimes curl inwards at the outer edges, which may almost meet in the middle line. The transfusion cells may extend to the outermost layer of the strand. In width of lumen they increase from within outwards ; while in length they vary from long narrow tubes to short cells whose length scarcely exceeds the breadth.

CONCLUSIONS.

Pherosphaera Fitzgeraldi recalls familiar European shrubs and trees growing in peat-bogs, at alpine altitudes, or in arctic regions, both as regards the construction of its leaves, including the xerophytic epidermis and hypoderma associated with very loose mesophyll, and as regards the narrowness of the annual rings. While the dominance of thick-walled tracheides in the stem is paralleled by similar tendencies in species of *Pinus*, *Larix*, and *Picea* when grown at considerable altitudes in Europe, yet the structure of the leaves is not widely different in design from that displayed by various species of *Pinus* and *Juniperus* of more low-lying sites.

The cause of these anatomical features of *Pherosphaera Fitzgeraldi*, which can grow in a soaking habitat, demands local investigation. Although the Tasmanian species is described as an alpine shrub, such does not appear to be the case with this species. For Dr. Stapf points out that the highest parts of the Blue Mountains, where the plant grows, are clad with Eucalypti, and that the Katoomba plateau (where the specimen described in Hooker's 'Icones Plantarum' was obtained) has a general elevation of 3,000 to 3,500 ft.

The Morphology of *Phylloglossum Drummondii*, Kunze.

BY

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With five Figures in the Text.

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I. INTRODUCTION.

PHYLLOGLOSSUM Drummondii was described for the first time in 1843 by Kunze, who regarded the genus as occupying a position intermediate between the Lycopodiaceae and the Ophioglossaceae, its supposed affinity with the latter family being founded on a superficial resemblance in habit to *Ophioglossum Bergianum*.¹ The following year Roeper associated *Phylloglossum* with the Lycopodiaceae, and in that family it has been placed by all later writers.²

While the anatomy and general structure have been dealt with by Mettenius,³ Bertrand,⁴ and Bower,⁵ no satisfactory conclusion has been reached in regard to the morphology of its annual tuber. Mettenius compared the yearly tuber with that of *Orchis* and the dropper of *Tulipa*, but, on the ground that these organs are structurally different, he maintained that the comparison was of little use. Bertrand described the anatomy of *Phylloglossum* in great detail, but he left the problem of the morphology of its tuber unsolved. Bower followed Treub in comparing the yearly

¹ Kunze : Bot. Zeit., 1843.

² Roeper : Zur Flora Mecklenburgs, ii, 1844.

³ Mettenius : Bot. Zeit., 1867.

⁴ Bertrand : Arch. bot. du Nord de la France, Nos. 30-34, 1884.

⁵ Bower : Phil. Trans., 1886.

growth of *Phylloglossum* with the embryonic form of *Lycopodium cernuum*, noting a resemblance between the tuber of the former, and the 'protocorm' of the latter.¹ His conception of *Phylloglossum* as 'a permanently embryonic form of Lycopod' received wide acceptance.²

Wernham, drawing an analogy between the tuber of *Phylloglossum* and the droppers of certain Monocotyledonous plants, suggested that like these it is partly foliar and partly axial. His investigation, however, was limited to two specimens.³

The morphology of the annual tuber of *Phylloglossum* is, then, an open question. Moreover, such solutions of the problem as have been put forward are hypothetical, and based on analogy rather than on a strict examination of anatomical structure. Since 'the question of the position of *Phylloglossum* chiefly turns upon the view we take of its annual tuber and its trophophylls,'⁴ it is of exceptional importance that its morphology be understood. When, therefore, by the kindness of Professor Benson a quantity of material was placed at my disposal, I decided to discover if a re-investigation of its anatomy might not throw light on the problem of its morphology. The result of this work is set forth in the present paper.

II. MACROSCOPIC STRUCTURE.

The general features of a fertile plant of *Phylloglossum*, at a stage previous to spore-dispersal, may be seen from Fig. 1. This figure was drawn from a specimen about $1\frac{1}{2}$ " high, bearing above the level of the ground five long tapering leaves, and a smooth cylindrical axis (P) terminating in a compact strobilus (S); while unbranched, horizontally running roots (R) and two tuberous bodies are buried in the substratum. The shorter and more slender tuber, Z, belongs to the growth of the present year, the other; T, dates from the previous season. The former, at the age figured, appears as a smooth exogenous outgrowth about $\frac{1}{4}$ " long, and slightly swollen at the free end, in which may be seen the outline of an enclosed bud, b. The tuber is an organ of perennation; it is set free at the end of the season by the decay of other parts of the plant, and after a period of rest germinates, the activities of the enclosed bud producing roots, leaves, frequently a cone, and always another tuber. These organs constitute the yearly growth of the plant. All that remains of the growth of the previous season is the torn sheath, sh, of the old tuber.

The general structure of *Phylloglossum* will be clearer, if reference be made here to the research of Professor Bower on the germination of

¹ Treub: Ann. Jard. Buit., vol. 8, 1889-90.

² For Bower's more recent views, see Presidential Address to Section K (Botany), British Association, Australia, 1914.

³ Wernham: Ann. of Bot., vol. xxiv, 1912, p. 335.

⁴ Bower: loc. cit., 1914, p. 6.

perennating tubers, by means of which he was able to trace the development of a new tuber, and the origin of the bud enclosed in its free end.¹ A new tuber appears first as a projecting mass of tissue with the growing point situated in a depression in the centre. The growing point, however, does not long maintain its central position, becoming first sunk, and then inverted owing to the rapid and unequal growth of surrounding tissue. Finally, it takes the form of a conical mass of meristematic tissue at the base of a narrow channel. This channel, although less distinct in older specimens, never becomes completely obliterated. Its position can generally be recognized, at least in transverse sections of a tuber, by the smaller cells which surround it. It had earlier been observed by Bertrand, and called the 'canal de Braun'.²

This protected position of the bud, and the fact that by intercalary growth it is carried some distance into the substratum, are features showing a marked specialization for a geophytic habit. The tuber can, however, be regarded also as a means of vegetative propagation, since more than one may be formed during the season.

In the course of this investigation two different collections of material were examined—the one which I owe to the kindness of Professor F. E. Weiss was obtained from New Zealand, the other was made in South-West Australia by Professor Benson in mid-winter, August 3, 1914. This collection consisted of over one hundred plants, about one-third of which were fertile, showing features similar to those of the specimen figured. Many of the sterile plants were very young, bearing only one or two leaves, but, like the fertile plants, each possessed one new tuber. The New Zealand specimens had evidently been collected later in the season, and, moreover, they were on the whole better developed plants. Five were exceptionally interesting in that each possessed two new tubers. These plants had been set aside by Professor Weiss for further investigation, and were with great generosity handed over to me.

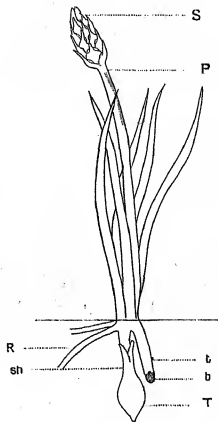


FIG. 1.

¹ Bower: loc. cit., 1886.² Bertrand: loc. cit., 1884.³ Thomas: Proc. Roy. Soc., 1901-2, p. 290.

III. GENERAL MORPHOLOGY OF THE TUBER.

The tuber of *Phylloglossum* has in turn been compared with the adventitious buds borne on the roots of *Ophioglossum*,¹ the tuber of *Orchis*,² the droppers of certain Monocotyledons,³ and the 'protocorm' of *L. cernuum*.⁴

The present paper seeks to bring forward anatomical data which throw new light upon the morphology of this plant. It is suggested that *Phylloglossum* is derived from some Lycopod possessing a branched vegetative system, which has become reduced and specialized in the adoption of a geophytic habit. The sharply defined pedicillate cone and the specialized storage tuber are advanced characters, and the juxtaposition of these with the frequent occurrence of feebly developed leaves and the absence of phloem from the stem is evidence that *Phylloglossum* is a reduced form. Examples of branching in *Phylloglossum* have hitherto been limited to a few isolated cases of dichotomy of the strobilus, but, as a result of an anatomical study, it is believed that branching occurs at least once in the yearly growth of every fertile plant, namely, on the formation of its annual tuber.

The details in vascular anatomy, upon which this view is based, will be dealt with under the following heads:

- (a) Stelar anatomy of a large fertile plant.
- (b) Origin, form, and course of the tuber stele in fertile plants.
- (c) Leaves connected with the tuber stele.

IV. VASCULAR ANATOMY OF FERTILE PLANTS.

(a) The Stelar Anatomy of a large Fertile Plant.

Considered generally, the most striking features in the internal anatomy of *Phylloglossum* are the frequently medullated protosteles, the mesarch position of the protoxylem, and the marked degradation of vascular tissue. The last point is illustrated by the almost complete absence of phloem, the breakdown of the protoxylem in the leaf-traces and in the stem, and the frequent occurrence of weakly formed tracheides.

In *Phylloglossum* the plan of the stele depends largely on the size of the plant and the number and position of tubers, leaves, and roots, and it is therefore not easy to give a general description. If *Phylloglossum* be a form which has suffered reduction relatively recent in descent, the largest and best developed specimens would be those in which the more primitive features might be expected to occur. It is

¹ van Tieghem : Recherches sur la symétrie de structure des plantes vasculaires. 1871.

² Mettenius : loc. cit., 1867.

³ Wernham : loc. cit., 1912.

⁴ Treub : loc. cit., 1889-90.



for this reason that a description of the best differentiated plant available is given first.

The plant in question is shown in Fig. 2, A. It bears on one side a large new tuber above which is situated a leaf, *sl*, somewhat shorter than the normal leaves of the plant (see p. 326). On the opposite side, two small protuberances may be distinguished, one of which represents an abortive tuber, *t'*, the other, *rl*, a much reduced leaf. The fact that there is in this plant an attempt to form a second new tuber will be dealt with later (p. 328); the importance of the plant in the present connexion lies in the anatomy of the well-developed tuber and its relation to the stele of the main axis. Before dealing with this, the general plan of the stele must be mentioned.

At the base of a fertile plant the entering root-strands unite to form a medullated protosteles, which, interrupted by frequent small gaps,

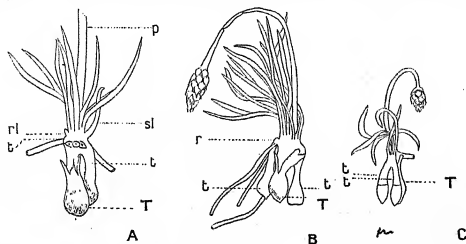


FIG. 2.

passes into the peduncle of the cone, and by repeated division supplies the sporophylls. The vascular supply of the new tuber is given off but a short distance above the level of the roots. The leaf-traces, which consist of a single mesarch strand of xylem, are often given off so near the base of the plant that they are in direct connexion with the root-strands.

The structure of the present specimen will be clearer if sections are described, proceeding upwards from the base of the plant. In Fig. 3, sects. 9, 8, and 7 (drawn with the Abbé camera), the entering root-strands are seen to unite to form the main stele, which consists, at this level, of anastomosing meristeles arranged round a central mass of parenchyma. In sect. 6, in position corresponding to the large central stele of previous sections, two steles are cut across, one consisting of a number of meristeles arranged roughly along two sides of a square, the other, a compact, medullated mass of xylem, with a well-marked gap, *g*, on the inner side. Both steles are associated with the exit of leaf-traces; in

the smaller stele one passes off from each edge of the gap. This stele is also connected with the vascular supply of a root. In the same section (sect. 6) the strand of the tuber, *t*, is seen, closely associated with

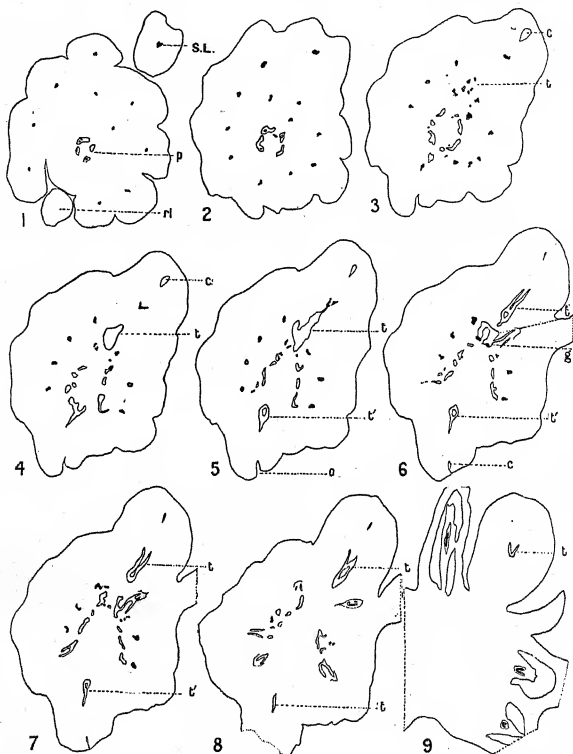


FIG. 3.

the compact central stele. Higher up the two are united, forming a solid mass of xylem, which is surrounded by the three leaf-traces, which emerged lower down, and a fourth, which is just being given off (sect. 5).

In higher sections this solid mass of xylem decreases rapidly in size (sect. 4), and finally dies out (sects. 3 and 2).

The bearing of these facts on the relation of the tuber to the main axis is clear from the vertical plan of the stele given in Fig. 4, A, which shows the relative position of the sections just described. On the right-hand side of the diagram is a large tuber, the stele of which makes a

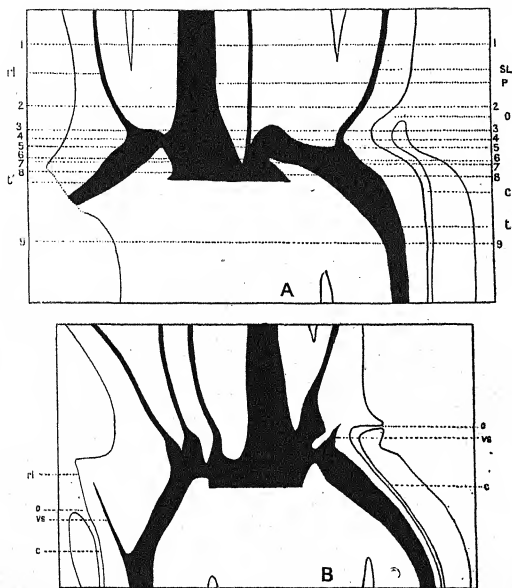


FIG. 4.

sharp upward bend before passing out and down the shaft of the tuber. The result is that sections below the bend cut the tuber stele in two places (sect. 6), while sections at a higher level cut the bend itself, and, therefore, show but a single mass of xylem (sects. 5 and 4). The collection of isolated tracheides in sect. 3 represents the highest part of the bend.

The most important features in the stelar anatomy of the plant considered may be summed up as follows: At the base of the plant

a medullated stele divides, each daughter stele showing a gap on the inner surface.¹ The smaller daughter stele, making a sharp bend, supplies the new tuber. The original gap is not continued over the bend, but it appears as the stele of the tuber passes out, giving it a characteristic U-form. From the tuber stele several leaf-traces pass out, constructed as those which arise from the main axis, and supplying leaves which number among those of the yearly growth.

The origin of the tuber stele from the stem, its structure, and its connexion with leaf-traces, point to one conclusion, namely, that it is morphologically a branch. In the following pages it will be seen if these features are sufficiently constant in fertile plants to justify this view.

(b) *The Origin, Course, and Form of the Tuber Stele in Fertile Plants.*

In the preceding section of this paper a plant was described, which is to a certain extent unique, at least in the material upon which this investigation was made. Its singularity consists, not so much in any particular feature, as in the fact that it combines several features of supreme importance in a morphological study. The aim of the present section is an examination of these features as they occur in other fertile plants.

Owing to the diversity in stelar anatomy which fertile plants show, it seems best to begin with a brief description of the tuber stele and its relation to the main axis in three distinct cases.

Fig. 5 gives a series of transverse sections of a large fertile plant with a single new tuber. Sect. 1, through the base of the peduncle, shows the stele as an almost continuous ring of xylem surrounding a relatively large pith. In sect. 2 this ring possesses a very distinct break at one side, the xylem being somewhat horseshoe-shaped, with cortex and medulla continuous through the gap. In the cortex facing this gap are three leaf-traces, which later become connected by tracheides with one another and with the edges of the gap (sect. 3), with the result that a tube of xylem is again formed of diameter nearly twice that of the ring seen in a higher section. This tubular condition lasts for one or two sections only, as the stele divides to form two arcs of xylem, one of which supplies the tuber, while the other breaks up to form the root-strands (sects. 4, 5, and 6). It is important to note that the arc which passes out into the tuber is that connected above with the three leaf-traces already mentioned.

The origin of the vascular supply to the tuber in this plant resembles, in several respects, that of the stunted tuber now to be described. We find, as before, at the base of the peduncle a ring of xylem, in which only small gaps occur (Fig. 3, sect. 1). Lower down a break appears on one side of the stele, giving it a horseshoe form in cross-section (sect. 2). Later

¹ Cf. the gaps occurring at each dichotomy of the axis in members of the *Lepidodendrene*, which possess medullated steles.

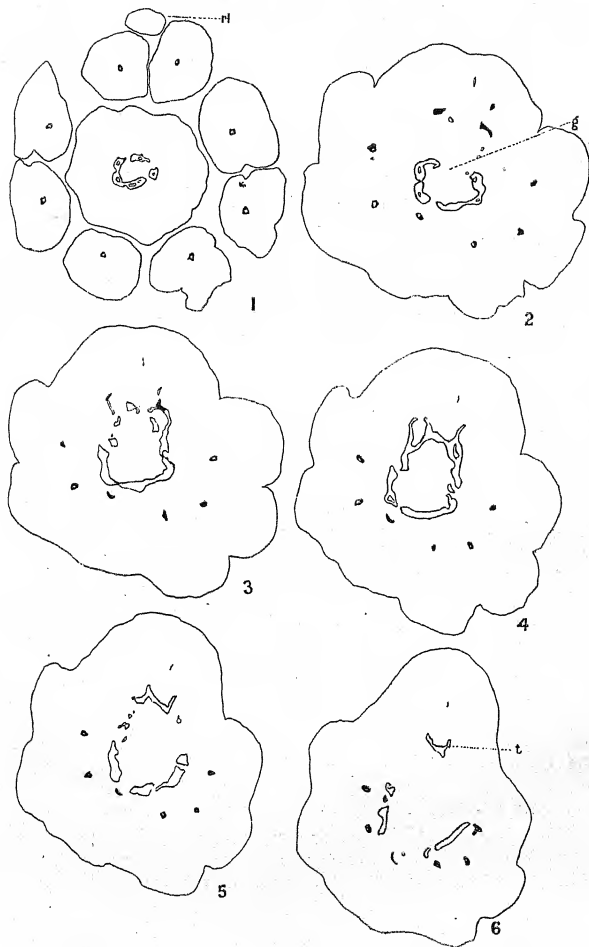


FIG. 5.

this gap is closed, in this case by xylem, which appears between the edges of the gap and connects the stele with a single leaf-trace (sects. 3 and 4). It is this part of the stele which supplies the strand for the new tuber. The difference between the tuber strands, as they leave the stele in the two plants, depends partly on the fact that the tuber is far better developed in the one case than in the other. Whereas, in the plant with a single tuber, the stele divides almost equally, in the plant now under consideration only a relatively small mass of xylem passes out to the stunted tuber. A difference in form may also be noted, the stele of the stunted tuber being from the first a compact strand of xylem with a small core of enclosed parenchyma.

The stele of one more fertile plant has yet to be described, since it is, perhaps, more typical of the smaller fertile plants in which the vascular tissue is less well developed. The plant in question possessed a cone, six leaves, and a somewhat slender new tuber. At the base of the peduncle two separate strands of xylem are found instead of a hollow cylinder as in larger plants. Lower down these increase in size, and a third strand appears, connected with a leaf-trace which joins the stele relatively early. The stele now shows a form roughly comparable with the horseshoe-shaped stele found at a corresponding level in larger plants, but it is less striking since the stele in the peduncle is already interrupted by large gaps.¹ In this plant no leaf-traces are connected with the vascular supply of the tuber. It arises from the medullated stele as a small arc of xylem, and, as it passes down the shaft of the tuber, it early becomes a slender cylindrical strand.

In two of the three plants just described, a distinct break in the stele of the main axis was observed immediately above the point at which the strand of the new tuber is given off. Though less conspicuous in the smaller fertile plants, owing to the weaker development of the stele, this break was found in eleven of the twelve cases examined, while the one specimen in which a gap was not found showed a thinning of the xylem in a corresponding position. Although this gap generally extends for a very short distance, the sections in which it does occur present a most characteristic appearance, and one which has been noted by previous workers. Thus Wernham concludes that the 'noticeable U-form of the upper part of the stem is, perhaps, the most striking feature',² while Jeffrey describes *Phylloglossum* as having 'a tubular stele, which, in the lower tuberous portion of the stem, constitutes in cross-section an almost continuous horseshoe of xylem'. He states also that 'the opening in the horseshoe corresponds to the outgoing strand, which passes into the resting tuber'.³

¹ The stellar gaps of *Phylloglossum* are not regarded as foliar gaps, though sometimes found in apparent connexion with leaf-traces (Jeffrey: Bot. Gaz., vol. xlv, 1908, p. 245).

² Wernham: loc. cit., p. 338.

³ Jeffrey: loc. cit., p. 244.

The fact that a gap is caused in the main stele by the exit of the vascular strand of the tuber has been used as evidence for regarding the tuber of fertile plants as morphologically a branch (p. 322). It is therefore of the greatest importance to find that the gap is such a characteristic feature in the anatomy of fertile plants.¹

Other evidence was found in the fact that the tuber stele of the plant first described makes a sharp bend as it passes out, thus suggesting such a change in the direction of growth of the organ as the branch theory of its morphology would involve (Fig. 4, A, *z*). In the majority of fertile plants this is not the case, for the tuber strand follows, almost as soon as it is free, a direct downward course, but stelar tissue is sometimes found above the level at which the tuber stele is given off (Fig. 5, sects. 2 and 3), and doubtless represents the definite bend seen in a better-differentiated plant. That the difference is chiefly one of degree may be seen by comparing the large tuber of Fig. 4, A, with the left-hand tuber of Fig. 4, B.

In this connexion it is also important to note in Fig. 5 how the form of the tuber stele changes as it passes out. When first free, it consists in cross-section of a horseshoe-shaped mass of xylem, with the arms of the horseshoe turned towards the main stele (sect. 4), but by the time it enters the shaft of the tuber (sect. 6) the curvature is in the opposite direction. The two positions of the gap in this single strand correspond to the two gaps seen in Fig. 3, sect. 6, where the stele of the tuber is cut in two places below the bend. We have, therefore, additional evidence that the tuber is an organ which, in the course of specialization, has made a definite change in its direction of growth. It is interesting that features similar to the above are shown by the steles of several other well-developed tubers.

Before leaving the subject of the course of the tuber stele, reference should be made to its behaviour as it traverses the shaft of the tuber. Not infrequently the tuber stele diminishes rapidly to a slender strand of tracheides, and dies out before the swollen end of the tuber is reached. In the larger specimens, on the other hand, the horseshoe form of the stele may be retained until it nears the swollen region, where it breaks up to form several separate strands. By further subdivision the tuber stele forms an irregular sheath of tracheides in the tissue surrounding the bud of the storage tuber.² These tracheides, which are little lignified and difficult to distinguish from the cells of the ground tissue, die out before the base of the bud is reached. Their presence in the largest tubers is

¹ The possibility of the gap being foliar, which was suggested by Wernham, is rendered still less probable by recent work, which dissociates very completely the Ophioglossaceae from the Lycopods. *Tmesipteris*, even if the stelar gaps are foliar, which is doubtful, is more commonly regarded now as showing Sphenopsid rather than Lycopsid alliance. Moreover, the 'organe de Mettenius', which Wernham suggests may be the vestige of a megaphyllous leaf is in reality a stage in the reduction of one of the normal leaves of the plant (p. 326).

² This sheath of tracheides was described and figured by Bertrand, loc. cit., 1885.

doubtless connected with the need for a more abundant water-supply. The more primitive features of the tuber would not be looked for in the long shaft of the tuber, which is clearly an adaptation to a geophytic habit, but in that part of the tuber most closely connected with the main axis. Such features have already been found in the origin of the tuber stele, and others will be dealt with in the following section.

c. Leaves connected with the New Tuber.

Mettenius, in his memoir of *Phylloglossum*, describes a small tongue-shaped body, frequently found above the new tuber. This structure he regards as an atrophied leaf, since, in his material, it was connected by transitional forms with the normal leaves of the plant.¹ A similar body was observed by Bertrand, and named the 'organe de Mettenius', but his material showed none of the stages which led Mettenius to his conclusion.² Bower, however, states that, in several of the mature plants he examined, a leaf placed above the new tuber was frequently smaller than the others, and he suggests that it corresponds to those structures observed in a similar position by earlier writers.³

The aim of this section of the paper is twofold; in the first place, to confirm the work of Mettenius by showing that the organ which bears his name is indeed a reduced leaf, and, secondly, to show how some of the lower leaves of the plant are connected with the new tuber.

The general structure of the tuber, the bud of which is sunk at the base of a narrow channel, has already been described (p. 317). The mouth of this channel is situated on the outer surface of the tuber, often at the base of a small hump of tissue borne in the angle between the new tuber and the peduncle of the cone. In some cases this hump of tissue can only be observed in a microscopic examination (Fig. 4, B, *r.l.*), in others it may be seen with the naked eye or with a lens, and recalls then the tongue-shaped body described by Mettenius and Bertrand (Fig. 2, *r.l.*). A vascular supply is generally found, consisting of a slender strand of tracheides, which dies out before the tip of the organ is reached (Fig. 5, sect. 1, *r.l.*, and Fig. 3, sect. 1). Occasionally, in place of this tongue-shaped structure a small but otherwise normal leaf is found, the 'supernumerary leaf' of Professor Bower. Such a leaf is shown in Fig. 2, *a, s.l.*, and appears in section in Fig. 3, sect. 1. A complete series of transitional forms connects such leaves with the various structures described above, and there is no doubt that all are merely stages in the reduction of one of the normal leaves of the plant.

¹ Mettenius: loc. cit., p. 99.

² Bertrand: loc. cit., 1885.

³ Bower: loc. cit., 1886, p. 670.

The occurrence of a stunted leaf in such a position is explained by its connexion with the tuber. Earlier in this paper (p. 324) reference was made to the fact that a leaf-trace, travelling in the cortex opposite the ramular gap, was connected with the stele of the tuber. Such a leaf-trace may belong to a normal leaf, or to a leaf in one of the stages of reduction just mentioned. So constant is this feature in fertile plants, that of fifteen tubers examined, only four were found unconnected with any leaf, and these were borne by small plants with relatively few leaves. Moreover, a tuber is frequently found bearing more than one leaf, as in the plant of Fig. 5, where the steles of two normal leaves and one which is much stunted are connected with the stele of the new tuber. Plant *b*, sketched in Fig. 2, was conspicuous both for the number of its leaves and for the fact that they were arranged unequally round the axis of the cone. It was found that from one tuber five leaf-traces were given off, from the other only two.

The leaf-traces are generally given off from the base of the tuber stele, that is, from the part near the parent axis, where the more primitive characters are likely to occur (p. 326), but in one case a small vestigial strand passed off from the tuber stele at some distance down the shaft (Fig. 4, B, *r.l.*).

The fact that leaf-strands pass off from the stele of the storage tuber, some of them supplying leaves which are quite indistinguishable from those borne by the main axis, confirms the view that the tuber of *Phylloglossum* is a specialized branch. Moreover, the stunted growth of certain leaves may be correlated with this specialization, since imperfectly developed leaves are always those connected with a storage tuber.

V. VASCULAR ANATOMY OF STERILE PLANTS.

The resting tubers of *Phylloglossum* always produce, on germination, one or more roots, a tuft of the characteristic cuneiform leaves, and a new storage tuber, but the spore-producing part of the plant may be absent. In these plants some of the leaves on the side from which the tuber is given off may be reduced, but the different stages in reduction observed in fertile plants were not all found. No sterile plant with a second new tuber has been recorded.

Serial transverse sections of a sterile plant show, in general, the following stelar structure: At the base of the plant the entering root-strands unite with the small strand of the tuber to form a stele, consisting of a core of xylem, which seldom shows a definite medulla. From this stele leaf-traces are given off, each consisting, as in fertile plants, of a single mesarch strand of xylem. As the leaf-traces pass into the cortex the stem stele diminishes in size, breaks up, and, before the leaf-bases are free,

completely disappears. This apparent disintegration and dying out of the upper part of the stele in sterile plants is the most striking feature in their anatomy, and one demanding an explanation. Earlier in this paper (p. 321) a plant was described in which the stele of a storage tuber made a sharp upward bend as it passed out, and this bend was taken as indicating a change in direction of growth. It may be noted that sections through this bend correspond very closely with transverse sections taken at a certain level in sterile plants, and the apparent dying out of the upper part of the stele in these plants may be due to a sharp bend in the axis. If this be so, sterile plants must consist of a slender unbranched axis, which bends over and forms the annual storage tuber. In support of this is the fact that Professor Bower, working on the ontogeny of the yearly growth of sterile tubers, identified the growing point of the new tuber with the apex of the stem itself.¹

In the largest sterile plant examined, the course of the stele is somewhat different from that described above, the chief difference being that it is for a short distance medullated, as are the steles of fertile plants, and the tuber strand, as it passes out, assumes the characteristic U-form. Moreover, the medullated stele shows a break which, corresponding in position to the tuber stele, recalls the ramular gap of fertile plants. The upper part of the stele is, however, apparently lost among the leaf-traces as in other sterile plants.

The U-form of the tuber stele, and the break which occurs in the stem stele above the level of its exit, are difficult to bring into line with the conception of sterile plants suggested above. They are explained, however, if, on analogy with fertile plants, it be assumed that branching has occurred on the formation of the new tuber, but that here the fertile branch has been arrested early in its development. It seems probable, therefore, that two conditions may exist in sterile plants: that a sterile plant may consist either of a simple axis, concerned only with the formation of a storage organ, or of an axis which has divided, one branch forming a tuber, the other being completely abortive, thus representing a condition intermediate between the small sterile and the simple² fertile plants. In either case the anatomy of sterile plants bears out the conclusion that the tuber of *Phylloglossum* is the specialized terminal part of a leafy axis.

✓ VI. BRANCHING IN PHYLLOGLOSSUM.

Phylloglossum has hitherto been regarded as a typically unbranched form of Lycopod, the rare cases of branching which have been recorded being restricted to the cone-bearing axis. Professor Thomas states that

¹ Bower: loc. cit., 1886.

² 'Simple' here refers to fertile plants with a single new tuber in which the axis is, therefore, only once branched.

about one plant in 2,000 may possess a forked strobilus, the two arms being equally developed.¹

If, however, we hold that the tuber is morphologically a branch, branching in *Phylloglossum* can no longer be regarded as a rare occurrence, since it happens at least once in the yearly growth of every fertile plant. Moreover, the New Zealand form not infrequently produces more than one new tuber during the season, and in such cases two acts of branching must have occurred.²

When two new tubers are formed they may arise on opposite sides of the plant, with the old tubers between them (Fig. 2, *b*), or near together on the same side (Fig. 2, *c*). In this case the habit of the plant suggests that the two tubers are the result of a dichotomy, and this is supported by its stelar structure. A medullated stele is found in the peduncle, and is later interrupted by a gap, below which, on the same side of the plant, a curved band of xylem passes out into the cortex. So far there is close agreement with the stele of a fertile plant with one new tuber: the difference lies in the behaviour of the branch stele. Whereas it normally passes, with some modification in form, down the shaft of a single tuber, in the plant now under discussion it gives rise to two smaller strands, and thus supplies two tubers. The daughter steles are slender strands, showing no characteristic gaps, since the division takes place when the original stele is an irregular curved band of xylem. The duplication of tubers in this way is consistent with the conception of the tuber as a specialized part of the axis, and affords an example of double branching in *Phylloglossum*.

Double branching also occurs in the axis of plants bearing a new tuber on two opposite sides. The anatomy of one of the three plants showing this feature has been fully described (p. 318), and, since the others agree with it in general structure, it is not necessary to do more than refer to the plan of the stele.³

A large stele is formed at the base of the plant by the entering root-strands. This divides, and the smaller product of the division supplies one of the new tubers. The larger of the two steles divides again, a small strand passing out to the second new tuber, while a large medullated stele passes up the peduncle of the cone. The distance between the two points of branching is so short that the tubers may appear to arise at the same level. That this is not the case is shown by the stelar anatomy (Figs. 3 and 4, *a*).

In these plants the new tubers have been formed by two successive

¹ Thomas: loc. cit.

² Thomas states that, while plants with one tuber are still in the majority, two tubers are frequently formed.

³ In one case the axis apparently branches twice, but the stele of one new tuber is unconnected with the vascular tissue of any other part of the plant. This can only be regarded as an anomaly resulting from the reduction which the plant has suffered in the course of adaptation to its environment.

acts of branching at the base of the axis, which finally terminates in a cone. In the previous examples the duplication was due to a dichotomy of the branch, which more frequently forms a single tuber.

Thus, not only is branching found occasionally in the strobilus of *Phylloglossum*, but normally in fertile plants on the production of each new tuber. It can, therefore, no longer be said that *Phylloglossum* is characteristically an unbranched form.

VII. CONCLUSION.

The interest which has been manifested in the monotypic genus, *Phylloglossum*, since its discovery in 1843, has centred round its most characteristic feature, the annual storage tuber.

Treub saw in it a resemblance to the organ of *L. cernuum*, which he designated a 'protocorm,' regarding it as of phylogenetic significance.¹ In this way he initiated a confusion which has been at the base of our difficulty in interpreting the true morphology of *Phylloglossum*.

With the increase of detailed knowledge of the embryogeny of Lycopods, the inconstancy of the 'protocorm' has been more fully realized, and opinion, as to its primitive nature has been modified, the present tendency being to regard it as an 'opportunistic local swelling', of physiological rather than of phylogenetic significance.²

This new conception of the 'protocorm' has not, however, helped to elucidate the problem of the morphology of *Phylloglossum*, if the old comparison with the embryo of *L. cernuum* still be made. Except inasmuch as both the 'protocorm' of *L. cernuum* and the tuber of *Phylloglossum* are manifestations of the tendency to local swellings seen throughout the family, the resemblance between them is purely superficial.

The present work, by detailed anatomical investigation, has shown that the tuber of *Phylloglossum* is a highly specialized leafy axis, the terminal bud of which functions both as a means of vegetative reproduction and as an organ of perennation. Though different in appearance and structure, the tuber of *Phylloglossum* is comparable with the resting buds of *L. inundatum* and the 'tubers' of certain Indian species of *Selaginella*.³

The position which *Phylloglossum* has hitherto occupied in the family Lycopodiaceae is rendered less isolated, since it can no longer be regarded as typically unbranched. On the other hand, the marked geophytic specialization, the mesarch character of the xylem, and the medullation of the stele, together with long established custom, justify the retention of separate generic rank.

¹ Treub: loc. cit.

² Bower: loc. cit., 1914.

³ Bancroft, N.: Ann. of Bot., vol. xxviii, 1914, p. 685.

✓ VIII. SUMMARY.

I. That the tuber of *Phylloglossum* is, in fertile plants, a modified branch is supported by the following facts:

1. A gap is left in the stele of the main axis by the exit of the vascular strand of the tuber.

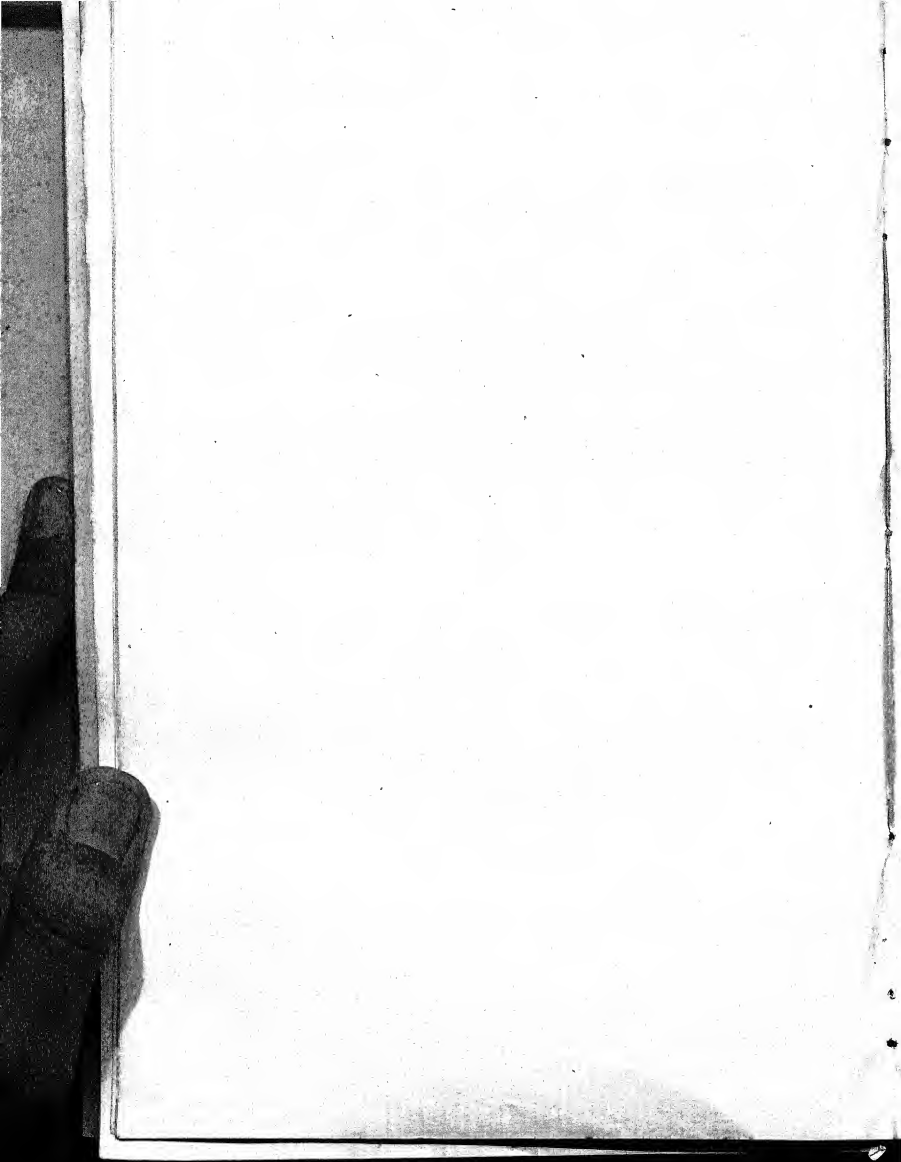
2. The stele of the tuber often shows a corresponding gap.

3. The tuber bears leaves, some of which are considerably reduced.

II. In general, sterile plants consist of a simple axis, the apex of which has formed a storage tuber. It is possible that, in the larger specimens, branching occurs as in fertile plants, but the arm, which in the latter produces a cone, is in sterile plants arrested early in development.

III. The tuber of *Phylloglossum* can no longer be compared with the protocorm of *Lycopodium cernuum*, but the two genera are brought nearer together, since *Phylloglossum* has proved to be not characteristically an unbranched form.

In conclusion, I wish to thank Professor Benson for suggesting this work, and for her helpful criticism during the course of the investigation.



The Physiological Anatomy of *Spartina Townsendii*.

BY

GEO. K. SUTHERLAND

AND

A. EASTWOOD.

With seven Figures in the Text.

INTRODUCTION.

SPARTINA is a small genus of very characteristic grasses, mainly natives of the Atlantic seaboard of America, where they are to be found abundantly in salt marsh and estuary. *Spartina cynosuroides*, the freshwater Cord Grass, penetrates inland to the Missouri River, and in the Western States it forms a large part of the grass of sloughs and wet marshes. In Europe only four representatives occur, if we regard *S. Townsendii* and *S. Neyrauii* as one species.

S. juncea is restricted to the western portion of the Mediterranean, whither it was introduced probably by shipping. Of the other three the oldest known is *S. stricta*, which Stapf regards as undoubtedly indigenous. It has the widest distribution of the European forms, but, notwithstanding its long establishment, it is becoming scarce on the south coast of Britain owing to the rapid spread of a later species. *S. alterniflora* was recorded first from the neighbourhood of Bayonne at the beginning of last century, and later it was discovered at the head of Southampton Water, down which it spread until its progress was checked by the remaining species, *S. Townsendii*, about whose origin and first appearance considerable uncertainty exists.

In his Flora of Hampshire, published in 1883, Townsend gives the first record of this species as 1878, when it was collected in the neighbourhood of Hythe, Southampton Water, by the brothers Groves, who described it shortly afterwards as a distinct species. But there is no doubt that it existed earlier, although overlooked. A specimen in the Warner Herbarium at University College, Southampton, collected near Hythe in 1870 and labelled *S. stricta*, is undoubtedly *S. Townsendii*. This carries it definitely

back to that date, but there is reason to believe that it occurred even before that time. The older accounts of the Spartan grasses vary much, and in Sowerby's 'Grasses' (1861) the opinion is expressed that the plants of *S. alterniflora*, collected near Southampton, were so like *S. stricta* that they could not be regarded in any other light than as intermediate varieties. But *S. stricta* is a fairly constant species showing remarkably few variations. Therefore the probability is that the doubtful specimens were in reality plants of *S. Townsendii*, which in many of its characters is intermediate between *S. stricta* and *S. alterniflora*. Unless the plants were examined carefully *in situ*, and at the flowering season, the appearance of this new plant might be overlooked for several years, mixed as it was with a very similar species, and growing in places not readily accessible. This view would help us to understand better its present extent and profusion.

Townsend's Spartan grass has been characterized by its phenomenal success on the shelving mud banks along the entire western shore of Southampton Water, whence it has spread with amazing rapidity over the available and suitable mud flats between Selsey Bill and St. Alban's Head, which form the natural boundaries of the sunken valley of the old Frome or Solent River. Here the numerous creeks and estuaries, harbours, and salt marshes from Poole to Chichester are well protected, while tertiary formations have supplied abundant mud. Again, the various stages of the progress eastward and westward have been short and favoured by eddying currents, which have helped largely in fruit dispersal. On both sides of these limits, where chalk ridges reach the sea, there are extensive stretches of shingle beach and cliff, broken by few suitable openings, with the result that for the time being the natural spread seems checked.

The value of this grass in fixing and in reclaiming shifting and unsightly mud banks has been recognized, and already attempts have been made to utilize it. Plants placed in the Medway have made considerable progress; others have also become acclimatized at both Blakeney Point and Wells Marsh in Norfolk. More recently the experiment has been extended northwards to the mud flats of the Forth and Don mouth.

At the present time it is the dominant species in the south of England. *S. stricta* survives in a few quiet backwaters, while *S. alterniflora* is disappearing fast before its more vigorous competitor, whose adaptation and success may be gauged by a glance across the Spartan beds from Cadland to Calshot, or from Lymington Harbour to Hurst Castle Bay.

It is inevitable that such an extensive and thick vegetation should affect the deposition of silt near river mouths, and hence tend towards a quicker levelling up. At present there exist no reliable data with regard to the rate, but it is hoped that a series of accurate survey measurements may be made at different stations along Southampton Water.

Apart from its usefulness, the grass presents interesting ecological features, and the present study was undertaken in the hope that it would throw some light on its adaptation to a life of periodic submersion, as well as form an introduction to a contemplated series of experiments on its physiology. The distribution and spread of the plant has been treated fully by Stapf, who is still carrying out his observations on its ecology.

EXTERNAL MORPHOLOGY.

Rhizome. Spartan grass owes its power of rapid extension mainly to its characteristic rhizomes, which vary in length from a few inches to over a foot, rarely exceeding one-quarter inch in diameter. Their length is dependent for the most part on the type of soil and the available space. They travel horizontally through the mud at a depth of from $2\frac{1}{4}$ to 4 inches, but frequently in the young stages they show positive geotropism and penetrate downwards for a short distance. Near their point of origin they are firm, with short hollow internodes. The greater portion of their length, however, is soft and flexible, the whole structure being adapted for penetrating a soft substratum. Short scale leaves are found in the bud stage, but the mature rhizome is invested merely by colourless sheaths devoid of blade and ligule. After proceeding for a short distance, rooting more or less freely at the nodes, the tip turns upwards and gives rise to aerial branches. New rhizomes are produced sympodially, and in the early stages there is nothing to distinguish them from the aerial shoots, except that the latter remain for a longer time within the sheath and are given an upward tendency. The creeping axes are shorter in a stony substratum, and hence it may also be that the question of the available space plays a part in determining the tendency and consequently the ultimate formation of culm and rhizome.

Culm. The erect cylindrical aerial axis, covered almost entirely by numerous investing leaf-sheaths which enhance its rigidity, reaches a height of from 2 to 4 feet. The first shoot arising from the upturned rhizome tip is usually the dominant one. Secondary culms spring distichously from its basal portion. Of these the lower and outer generally grow more quickly, checking the development of the higher ones. Only at the margin of the clump or belt is it possible for plants to develop all or most of their aerial shoots.

Leaf. The leaves are also distichous. The first three or four are practically scales. As soon, however, as the axis rises above the ground short green-tipped blades appear, increasing in size as the stem is ascended, until they reach a length of twelve to eighteen inches. The lowest blades are thrown off at an early stage, separating at the articulation. This helps to distinguish this species from *S. alterniflora*, in which the lower blades are retained longer and wither gradually. A short soft-celled ligule, tipped

with hairs, encircles the stem and prevents water or mud from lodging between it and the sheath. The long smooth pale-green sheath clasps the axis firmly, completely surrounding it for the greater part of its length and passing into the blade through the pulvinar articulation. The lower blades form angles of from 45° to 60° with the stem, while the upper ones are more erect. The angle increases with submersion. During the fall of the tide the leaves swing up and down in the surface film until they are suddenly released and spring into position.

The adaxial surface of the blade is increased enormously by from forty to fifty ridges and furrows running from just above the pulvinus to the apex. These provide an increased assimilatory surface in addition to protection for the stomata. The papillae and waxy coating on this surface give it a velvety and glaucous appearance.

Root. The roots are adventitious and divided into two distinct sets. One type is long, relatively thick, smooth, and practically devoid of branches. This fixing form grows normally to a depth of six to twelve inches, serving to anchor the plant firmly in the soft mud. While its root-cap is large, root-hairs rarely, if ever, occur. The other type is shorter, thinner, and much branched, possessing a poorly developed root-cap and very sparse root-hairs on the youngest branches. Generally this latter form springs from, and clusters round the basal joints of the aerial axis, forming a densely woven, horizontal matting which aids largely in transforming the soft mud banks into comparatively firm turf. Similar tufts of branched roots spring from the rhizome nodes also. Their function is mainly absorptive. Both kinds of roots frequently show negative geotropism, probably an adaptation to the continuous silting.

ANATOMY.

Epidermis. The adaptation of the plant to alternate aerial and aquatic existence is marked by peculiar and special epidermal structures wherein it differs from any other grass genus examined. The two most striking features are the very distinctive hydathodes and the forked accessory stomatal papillae, both of which are undescribed as far as we can ascertain.

A distinct waxy coating covers all exposed portions above ground, giving the glaucous appearance. This is most pronounced on the adaxial surface of the leaves, where the epidermis is thinner, shows less cutinization, and is covered by numerous papillae as in Fig. 1, *r*. These conical or peg-like projections also show little cutinization. On the narrow cells of the ridges they run along in pairs which frequently become coated over in the old fully developed leaves, as in Fig. 1, *r*, *pa'*. This adds to the protection of the most exposed portions. The epidermal cells along the sides of the grooves are wider, and the number of papillae consequently greater. These, however, undergo no change. The papillae aid the waxy

coating in preventing water from adhering to, and wetting the upper surface. Their efficiency may be gauged by the fact that leaves, submerged in the laboratory for more than twenty-four hours, were quite

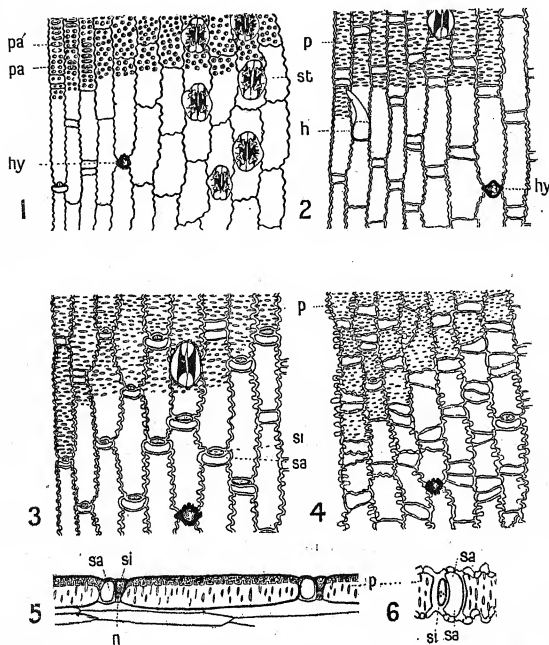


FIG. 1. 1. Epidermis of upper surface of leaf-blade, showing simple papillae (*pa* and *pa'*); hydathode (*hy*); and special stomata (*st*). 2. Epidermis of abaxial surface with ordinary stomata; pits (*p*); and hair (*h*). 3. Epidermis of abaxial surface of leaf-sheath immediately below the articulation: silica-cell (*si*); saddle-cell (*sa*). 4. Epidermis of articulation region, showing thick-walled pitted cells with very wavy outline. 5. Radial longitudinal section through 3: nucleus beneath silica body (*n*). 6. Enlarged surface view of the two types of short cells.

dry when taken out and shaken slightly. The normal period of natural submersion rarely exceeds a few hours.

The number of variations in the epidermis of different portions of the plant are merely changes rung on an essentially simple ground-plan, either of long cells alone, or more often of long cells alternating with

short ones, singly or in pairs. Their radial walls may be straight, but more frequently they present an undulating margin, most pronounced in positions of great strain like the pulvinar region, as in Fig. 1, 4. The inner surface of the sheath, protected by being pressed firmly against the stem, has thin, straight walls, while the outer epidermis (Fig. 1, 3 and 5) is coated with a thick development of cuticle. The adaxial surface of the blade is cutinized slightly, whereas the abaxial side has a strong, resistant layer which not only protects the mesophyll, but adds materially to the rigidity of the leaf. The poor development of this coating on the upper surface is compensated by the protection afforded by the furrows, and by the curling of the leaves when the water-supply is limited or transpiration excessive.

Long cells. These occur alone only in protected regions like the inner epidermis of the sheath and along the sides of the leaf-grooves. In most other parts they alternate with short cells, and possess strongly thickened outer and radial walls with numerous round or elongated pits (Fig. 1, 2, 3, and 4). Over the articulation between sheath and blade the thickening is most pronounced, forming strong radial flanges or girders between elongated large pits (Fig. 6). Freedom of movement at this point is facilitated by the shortened cells, whose folded fan-like walls (Fig. 1, 4) are capable of lengthening with a kind of bellows-action.

The most interesting of these long cells are the *motor-cells* (Fig. 4, 2, *m.c.*), which were first described for grasses by Duval-Jouve, who regarded them mainly as silica-containing cells, to which he gave the name '*cellules bulliformes*'. In *Spartina Townsendii* they form belts three cells wide running along the bottom of the furrows of the blade. They are clear and colourless, containing little solid matter but abundant water easily given up. While their inner and radial walls are very thin and collenchymatous, their outer walls differ only slightly from the adjoining epidermal cells. They are well developed towards the middle of the blade, being much deeper than the other cells along the furrows, but towards the margin little difference is seen. This accounts for the leaves rolling up completely only when excessive drying takes place.

Short cells. These show greater variation and are of two distinct types, one containing no silica or only traces, the other with a relatively massive, definitely shaped silica body. The former, which occur singly or in pairs between long cells, may be regular, but frequently they become saddle-shaped, cruciform, biscuit-shaped, or even dumb-bell-like (Fig. 1, *sa*). They invariably show less thickening than the adjacent long cells, and no pits are present on their outer walls. More interesting, however, is the second type containing a distinct silica mass. Each is accompanied by a short cork-cell placed always on the side towards the base of the plant, and forming a kind of saddle, in the hollow of which

the silica-cell lies, partly embraced by the upcurved ends. This is shown in Fig. 1, 3, 4, and 6. This type appears wedge-shaped in longitudinal sections of the leaf. The blunt end is outwards (Fig. 1, 5, *st*) and covered by a very thin wall, difficult to distinguish owing to its delicate structure and the refraction of the mass beneath. The silica body occupies the greater part of the cell. It is invested by a thin layer of protoplasm and blocks the entire upper and wider end of the cell cavity by which its outline is determined. It is easily recognizable both by the presence of small included air-bubbles, and by its resistance to stains. These cells develop from ordinary short, rectangular ones, and are most abundant in the upper outer epiderm of the sheath, where, immediately below the articulation, they equal the long cells in number. They cease at the point

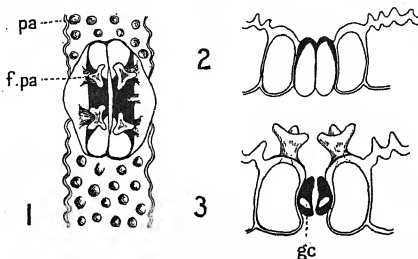


FIG. 2. 1. Surface view of special type of stoma with forked papillae (*f.pa*); simple papillae (*pa*). 2. Transverse section through swollen vesicular ends of guard cells. 3. Transverse section near the middle and thickened portion of guard cells (*gc*).

where the break appears later. A few occur along the ridges of the blade and on the peduncle, but none have been found on the rhizome, the stem, or the first-formed leaves. Their distribution lends strong support to the view that they add to the rigidity of the plant, which is aided largely by the closely investing leaf-sheaths, on whose outer surface they are most numerous.

Stomata. The stomata belong to the characteristic grass type. The walls of the middle portions of the guard cells are so thickened that the cavities connecting the swollen ends are reduced to narrow passages, as in Fig. 2, 3. The slit is slightly longer than these rigid bridges, which are carried bodily apart by the swelling of the vascular thin-walled ends (Fig. 2, 2).

The most active stomata are those situated on the adaxial surface of the leaf over the loose chlorenchyma, where they are more numerous than in any other part of the plant. There they occur in two, rarely

three, close rows on each side of the laminar furrows. These rows (Fig. 4, 2) are about two cell-widths apart, and almost corresponding distances from the motor-cells forming the bottom of the groove, and the line of hydathodes nearer the angle of the ridge. The stomata are of exceptional interest on account of the unique structure and placing of the papillae on the subsidiary cells. The papillose epidermis of this surface has been noted already. There are two massive papillae on each subsidiary cell, placed opposite the end of the stomatal slit (Fig. 2, 1 and 3). These expand at the top into two, three, or more rarely four, short branches which are strongly lignified, like the thickened walls of the guard cells. Frequently there is a simple papilla between them, corresponding to the middle of the pore. All bend over the guard cells, forming a fringe round and over the stoma as in Fig. 2, 1.

A small piece of leaf immersed in water showed a tiny air-bubble captured by these furcate papillae. Doubtless when the leaves are submerged the entangled air-bubbles prevent the entrance of water through the slits, and in this way the most active stomata are prevented from admitting water into the air-spaces at a time when they are open or partly so. This apparatus, along with the simple papillae and the waxy coating, goes a long way towards an explanation of the plant's adaptation for its dual existence.

The normal type of stoma occurs sparsely over all chlorenchyma. They are abundant on the inner epiderm of the sheath, but then it presses so tightly against the stem that no water gains entrance. Although their appearance on the rhizome sheaths is more surprising, the absence of chlorophyll prevents the manufacture of osmotic substances, and consequently the guard cells are inert.

Hydathodes. Hydathodes of a type apparently hitherto undescribed take the place of the water pores found in many submerged plants, being distributed widely in definite tracts in the active chlorenchyma, usually near large water-storing cells. On the upper surface of the leaf they are arranged in a row (Figs. 1 and 4) along each side of the furrow, about two or three cell-widths from the ridge angle, and one or two from the upper line of stomata. Here the epiderm consists of long cells alone, and the hydathodes are placed between every two or three of these in longitudinal series. On the abaxial surface of the leaf-blade and sheath they form a similar line in the large-celled tissue between the sclerenchyma bands over the bundles. They are absent from the rhizome, the invested portion of the stem, and the inner surface of the sheath, but occur abundantly on peduncles, and even glumes in the more-pronounced assimilatory bands, although always near fairly large cells with watery content. They are placed invariably between the ends of two long cells which become slightly narrower as they approach one another (Fig. 3, 1). However, instead

of meeting their corners project outwards again and meet the walls of the lateral cells, forming a cylindrical cavity whose rim in surface view is very thick, with the inner side smooth and circular, while the outer is wavy, varied, and even pitted (Fig. 3, 1). The hydathodal space is bounded therefore partly by four cells, along whose lines of contact are four vertical ridges or flanges, with the intermediate portion facing each cell thinner walled.

A transverse section of the leaf shows a flask-shaped organ, as in Fig. 3, 2, with the neck projecting into the epidermal cavity described, and the swollen basal portion embedded in the underlying tissue; a radial longitudinal section differs inasmuch as the basal part is elongated and boat-shaped, as in Fig. 3, 3. The cap portion of the hydathode has a distinctly stratified, mucilaginous wall, fitting the cavity tightly yet free from its walls. Although in the adult plant it is usually on a level with the surface or slightly below it, in the very young stages it projects a little distance beyond. It rests on a strongly lignified and cutinized collar (Fig. 3, 2, *c*) which marks the region where the thin-walled swollen base abuts on the retreating sloped lower corners of the adjacent epidermal cells. This collar is so strongly developed as to give the impression of a thick partition at this point. This is accentuated by a slight projecting ridge on its inner upper surface.

The basal portion of the hydathode is elongated parallel to the axis of the plant and has pointed pyramidal ends. It is thin-walled and densely filled with protoplasm, in the centre of which lies a relatively large nucleus. In addition to shape, it is sharply marked off from the surrounding cells by the absence of chloroplasts. The protoplasmic content of the cap is also dense, and stained sections give the impression of its being connected through the narrow neck by numerous strands of protoplasm to that in the lower part. A nucleus has also been observed in the cap, placed sometimes near the tip, sometimes partly hidden by the collar. The more frequent occurrence of nuclei in the cap portion in younger material would strengthen the view that the hydathode consists of two cells whose common wall has been resorbed at an early stage.

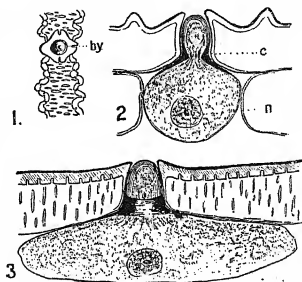


FIG. 3. 1. Surface view of hydathode (*hy*), from abaxial surface of leaf. 2. Transverse section of leaf showing hydathode: (*n*) nucleus; (*c*) collar. 3. Radial longitudinal section of abaxial surface of leaf, showing hydathode.

In very young leaves the tip of the hydathode projects a short distance above the surface like a swollen glandular hair. This suggests a possible theory as to its ontogeny. The hairs so abundant in many land grasses would be useless to the plant when submerged. This is partly borne out by the fact that many hairs drop off before the leaves unroll and open. Then the hairs in *Spartina* correspond in position to the short cells between two long ones, just as the hydathodes do, and occasionally they have been observed occurring along the same lines as the latter in young plants. It is possible, therefore, that these excretory organs are really hairs modified to meet a new set of conditions. An extensive examination of the genus is necessary before a definite statement can be made with regard to the point.

These hydathodes differ from any described forms, and are certainly unique in grasses. The nearest approach to them are the secretory cells, discovered by Sauvageau in aquatic Monocotyledons, and described by him in various papers on the structure of the leaves of these. In *Cymodocea aequora*, for example, they are distributed for the greater part along the margins of the leaves; in others they may be scattered irregularly over the surface. These, however, are merely larger epidermal cells whose outer walls remain thin, and become distended and convex, while the inner portions penetrate slightly into the mesophyll. They resemble those described above in function, but lack their more definite structure.

The *Spartina* type of hydathode may undoubtedly be regarded as a kind of safety-valve for getting rid easily and quickly of excess water and mineral salts, both of which, in abundance, are accessible, as a rule, to the plant. Thus fairly rapid loss of these is not dangerous to it, a fact which helps to explain the absence of any kind of epithem acting as a filtration-tissue, as in so many Dicotyledons. This want, common curiously enough to most Monocotyledons, facilitates rapid exudation. Their activity in this respect may be demonstrated very simply by cutting some plants and placing the cut stems in water under a bell-jar. In a few hours an immense number of drops may be seen on blade and sheath. The hydathodes forming the lines along the furrows function so quickly that tiny sparkling drops may be detected in less than an hour. These are much more active than those on the abaxial surface, owing doubtless to their greater proximity both to the large water-storing cells and the special assimilating tissue.

Large quantities of salts, especially sodium chloride, are present in the excreted water. These may be detected by chemical methods in the drops given off, but a more striking demonstration of their presence can be seen in nature. While examining some plants, whose upper leaves and peduncles had been exposed continuously for some days of neap tides to a fairly dry atmosphere, our attention was directed to numerous, small, white, worm-like

castings scattered over these parts of the plants. These were often from 1 to 2 mm. long, occurring over the hydathodal lines and only on the living parts. On examination they proved to be heaps of cubical crystals, mainly of sodium chloride. Rapid evaporation after exudation had enabled the salts to crystallize out, and their accumulation in such castings had been favoured by a continuation of dry weather and the failure of the neap tides to submerge the upper portions of the plants for a few days. The worm-casting-like form of the heaps of crystals, their distribution on the plant, and their absence from withered or dead portions prove conclusively that they were products of excretory activity, not the remains after evaporation of clinging drops of water or spray. What is most surprising is the amount of salts excreted by these small structures.

A shoot of *Spartina* with several leaves, when cut off under water and then transferred to a bell-jar with the cut end dipping in water, shows practically as great an excretion as potted plants placed in similar atmospheric conditions. The cut ends of the vessels are immersed freely in the water, continuity with the supply being secured without the intervention of an active cortical tissue as in the roots. The position of the most active of all the hydathodes in the stellate spongy chlorenchyma, with a thin layer of the same between them and the water-storing cells, precludes the possibility of much local pressure outside the hydathodes themselves. This would seem to throw the onus of their activity upon their own structure and contents, inducing the belief that the excretion is due mainly to some form of protoplasmic activity.

When the plants are submerged, some such system is necessary to get rid of excess water and salts, and prevent flooding of the air-spaces. A similar danger must be met even when they are exposed by the fall of the tide, for the cushion of air, held by the dense vegetation belt over the moist substratum, is more saturated than the atmosphere immediately above, with the result that transpiration is lessened and an auxiliary method of water-excretion rendered necessary. This is found in the system of hydathodes, which also enables the plant to throw off immense stores of salts.

Rhizome and Culm. Both the underground and aerial shoots conform to the usual grass type. One distinctive feature, associated with the habit of the plant, is the development of air-passages which in the former are large, and separated by radial parenchyma plates only one or two cells in thickness, while in the latter they are smaller and separated by much thicker radial walls. Usually they arise by the development of stellate tissue, and its subsequent disruption; sometimes large rounded cells break down without any appearance of stellate cells.

Leaf. The leaf also shows few variations from the normal furrowed grass type, except in the epidermal characters already described. Forty to

fifty fibro-vascular bundles, one for each ridge, run up through sheath and blade. They are of two kinds (Fig. 4, 1 and 2), alternating with one another. Round each bundle are two rings of cells. The inner consists of smaller cells, regular and very strongly lignified in the larger type, less regular and

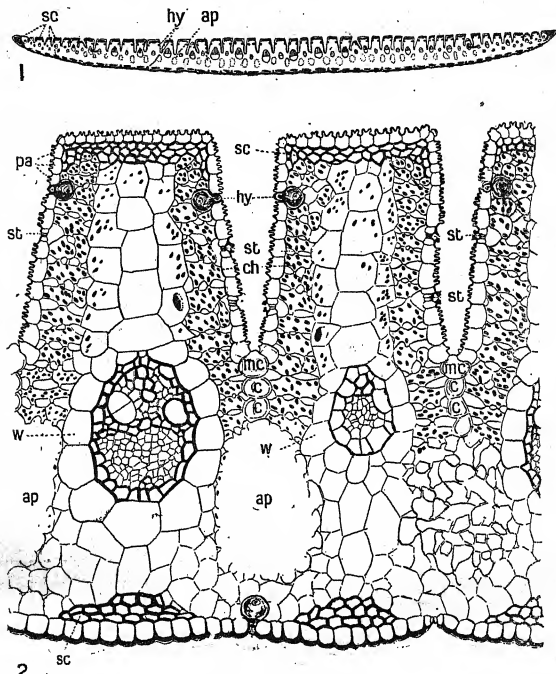


FIG. 4. 1. Transverse section through leaf-blade, showing the distribution of hydathodes, air-passages, and sclerenchyma. 2. Portion of 1 very much enlarged: (*pa*) papilla; (*st*) stoma; (*sc*) sclerenchyma; (*hy*) hydathode; (*ch*) chlorenchyma; (*mc*), motor cell; (*c*) partition cell; (*w*) water-storing envelope; (*ap*) air-passage.

only slightly thickened in the second; the other envelope consists of large thin-walled elongated cells. In the young plants they contain chloroplasts, which, as growth proceeds, disappear first from the upper cells, then from those towards the lower surface, and finally from the lateral ones. These

large clear cells (Fig. 4, 2, *w*), from their watery contents and proximity to the bundles, may be regarded as a kind of transfusion-tissue, or water-storage system. Two or three rows of similar large cells extend from this vascular sheath and unite with the subepidermal stereome. These at first also contain chloroplasts, which gradually diminish or even disappear finally. Ultimately these cells have a large water-content. They compose the main part of the mesophyll bridges supporting the bundles, and by their turgidity, combined with the sclerenchyma strands, form girders strong enough to keep the leaf erect even when unrolled.

The chlorenchyma consists of irregular stellar cells, curiously elongated like a palisade layer, with radiating lateral arms and large intercellular

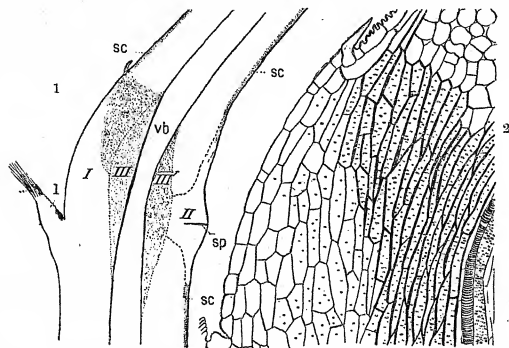


FIG. 5. 1. Longitudinal section through junction of leaf-blade and sheath: (I) parenchyma cushion; (II) thickened pad of articulation with split (*sp*); (III and III') sclerenchyma sheath surrounding reduced vascular bundle; (*l*) ligule; (*sc*) sclerenchyma; (*wb*) vascular bundle. 2. Enlargement of regions (I) and (III), showing type and distribution of supporting tissue, and pitting. The large colourless soft-walled cells under the convex thin-walled epidermis represents the active part of the pulvinus.

spaces, as in Fig. 4, 2, *ch*. It extends along the furrow to a little way below the bottom of the groove, between the water-storage ring and a short median line of two or three circular cells (Fig. 4, 2, *c*), whose walls approach collenchyma in structure, in this respect corresponding to inner walls of the motor-cells below whose median line they lie. The partition cells probably both help to strengthen the hinge arrangement and aid the motor-cells to absorb and to give up water. They terminate on the air-passages which run between the bundles through sheath and blade.

The air-passages arise from blocks of rounded cells with few or no chloroplasts. The cells become stellate and finally collapse, forming the lacunae (Fig. 4, 2, *ap*), which are interrupted by transverse diaphragms.

These latter are most numerous in the sheath, where they can be seen readily through the epidermis, owing to the enclosed tubes of air.

The inner surface of the sheath is continued upwards in a short ligule (Fig. 5, 1), terminating in a brush of fine hairs. The solid portion consists of moderately thin-walled, slightly elongated cells. The surface next to the stem, to which it is applied closely, has a smooth epiderm like the inner side of the sheath, while that towards the blade consists of cells, as in Fig. 7, with the long diameter nearly at right angles to the surface, and their outer walls notched, uneven, and mucilaginous. As the leaf-base is not always at the same angle to the stem, it seems likely that this upper surface of the ligule acts as a pulvinus in preventing its being torn away from the stem. The large, clear, colourless cells of the whole structure are adapted for holding water, which is retained, even under unfavourable conditions, by the mucilaginous coating. In this more or less turgid condition the ligule is pressed tightly against the stem, preventing the entrance of either water or mud, while the clefts (Fig. 7, *cl*) at its base permit the blade to sway freely without jerking the ligule suddenly away.

Pulvinus and Articulation. Immediately above the insertion of the ligule there is a thickening of the blade, due to the greater development of the mesophyll at that point. Under this short, convex stretch of epiderm, which shows little cutinization or thickening, lies a mass of soft-walled cells with slight almost invisible pitting. In longitudinal section they present the outline shown in Fig. 5, 2. Beneath this cushion the cells become thickened, distinctly pitted, twisted, and fibrous, forming a solid sheath round the bundle, which is slimmer at this point, and minus the water-storing envelope. This investing mass of mechanical tissue is thickened near the middle of the pulvinar region, from which it thins away upwards and downwards, as in Fig. 5, 1, III and III'. The sclerenchyma strands under the epidermis of the ridges above this region pass in under the soft tissue to meet the central supporting mass, while those under the abaxial epidermis stop short where the articulation bulges out on the dorsal side. This joint (Fig. 6) is marked by a darker, more glossy surface, where the epidermal cells are short, with thickened pitted outer walls and very wavy radial ones. Beneath this bulging portion is a pad of thick-walled cells with large pits; on the inside it abuts on the central supporting sheath. Above and below are the ordinary mesophyll cells.

Transversely along the middle line of the abaxial surface of the joint a split appears later, cutting across the strengthened pad without any definite order. This split forms a pseudo-articulation on which the leaf-blade swings more freely for some time before it falls off.

The soft-walled tissue on the adaxial surface acts as a pulvinus. When the plants are submerged and contain abundant water, these cells are turgid and the leaf-blade is bent back, but when they are exposed to a drying

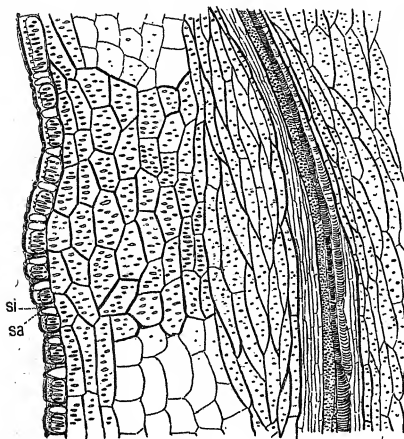


FIG. 6. Longitudinal section through regions (II) and (III') of Fig. 5, z, showing the thickened dorsal pad through which the split or pseudo-articulation appears later. The epidermis is strongly thickened and cutinized. Silica-cells (*si*), abundant on the upper part of the sheath, stop at the point of splitting. Saddle cells (*sa*) accompany them.

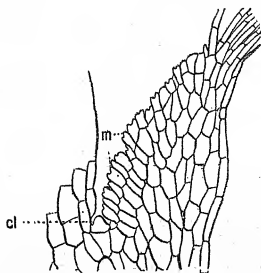


FIG. 7. Longitudinal section through the ligule with notched thin-walled mucilaginous cell (*m*); and clefts (*cl*).

atmosphere, part of the water is given up and the leaf becomes more erect, making it easier for the motor-cells to roll the leaf, and thus check transpiration from the upper surface. The latter danger is also lessened by a smaller surface being presented to the sun's action when the leaves are more upright.

Plants placed in water in the laboratory and then allowed to dry showed a pulvinar movement of ten to fifteen degrees. This is larger than occurs normally in nature. Freedom of movement at the articulation is facilitated by the thinning of the bundles there and by the migration of the stereome from a subepidermal position towards the centre, while sufficient mechanical support is given to the pulvinus by the thickened central sheath of sclerenchyma with its dorsal pad.

Fixing Roots. The fixing roots are much thicker than the absorptive ones and are never branched. The epidermis and the layer beneath it finally decay, so that the persisting exodermis represents the original third layer, contrary to the usual Monocotyledon rule. The layer next to the latter consists of smaller cells also sclerified. Then follow five or six rows of compact parenchyma, the outer cortex, ultimately, showing moderate sclerification. Cubical crystals occur in abundance in these layers. The inner cortex consists of a large number of rows of extremely regular parenchyma with rectangular air-chinks at every corner. Even in old fixing-roots the regularity and embryonic appearance of these cells are retained, so that the sections appear like the young roots of many Monocotyledons. Lacunae, of the type more common in the absorbing roots, also occur, either all along except at the tip and near the insertion, or at irregular intervals.

Absorbing Roots. At first these are anatomically the same as the young fixing roots, but are even then considerably thinner as a rule, owing to the smaller amount of cortex. In the adult roots all the inner cortex, except that at the actual growing-point, shows numerous well-marked radially elongated air-passages, stretching from the inner edge of the outer cortex to within two or three cell-rows of the endodermis.

The lacunae, so well developed in this latter type of root and locally in the former, arise as follows: A certain small number of radial rows of cells in the inner cortex show considerable enlargement. The cells of the neighbouring rows, not growing in size much and sometimes even collapsing, meanwhile become stellate, usually four-armed, and the intercellular spaces at their corners thus become enlarged. The radial walls of these rows of stellate cells become considerably thickened, and at points single cells or rows of two or three decay altogether, and only their radial walls plus small traces of their tangential walls attached to them persist round the gap. Each tangential wall becomes split across, and finally numerous narrow, complete radial rifts are formed in the inner cortex by the decay of com-

plete rows of stellate cells. Each space is always bounded by the intact radial walls which form limiting membranes.

The result is that the inner cortex has a small number of radial rows of large intact cells, separated by air-passages, in each of which occur several persistent radial membranes bearing slight vestiges here and there of the tangential walls of the defunct cells. These membranes never occur in the air-passages of the stem, rhizome, sheath, or glumes. Where the rootlets are connected with the absorptive root there is always a sheath of fairly compact, non-stellate cells left, about four cells thick, to surround the inrunning axis as it crosses the cortex.

The Inflorescence. The inflorescence of 3-13 somewhat spike-like axes conforms to the grass type, with one flower to each spikelet. These begin to form very early; although flowering does not usually begin until August and September, their component parts are well established by the end of May. Lodicules are absent and the glumes are compressed, both adaptations to the plant habit.

The surface and the contents of the versatile anthers are slightly mucilaginous as well as the plumose stigmas. The flowers are protogynous and wind-pollinated.

In connexion with the scarcity of hairs on the vegetative portions of the plant, already commented on, their abundance on the glumes is interesting. Of course the inflorescence is subjected to less submersion, but that alone seems an insufficient explanation. Some of the glume-hairs are very large, especially on the keels, and have groups of cells lying against their lower sides. As these latter have numerous pits on their outer walls and also on their inner, they can absorb water readily, and possibly alter the angle of the stiff hairs. In this way they might play a part in opening and closing the flowers. It is also probable that they hold air-bubbles in the spikelets when submerged.

SUMMARY.

Distribution. *Spartina Townsendii* probably occurred along Southampton Water considerably earlier than 1870—the date of the earliest recorded specimen in the Warner Herbarium. Its present natural distribution is limited by Selsey Bill and St. Alban's Head, the boundaries of the sunken valley of the old Frome or Solent River. At those points the chalk ridges reach the sea, and outside them lie fairly long stretches of shingle beach or cliff, broken by a few or no suitable estuaries or mud flats for a considerable distance.

External Morphology. There are two sets of roots. The anchoring or fixing roots are long, unbranched, devoid of root-hairs, and penetrate straight down into the mud; the absorptive roots branch freely, forming a dense, more or less horizontal web. Both types occasionally show marked negative

geotropism, and thus occupy newly deposited strata. The rhizome, whose length varies with the substratum, differs little from the ordinary grass type, as also the erect haulms. Rigidity is aided by the long-continued investment of the stem by the leaf sheaths. The long, rigid, semi-erect leaves belong to the furrowed type, rolling to a limited extent and showing a distinct pulvinar action at the articulation. The ligule also possesses a kind of pulvinus.

Anatomy.—The epidermis shows great local variations in the outline, arrangement, thickening, and structure of its units. Exposed portions have a distinct waxy coating overlying thickened and cutinized walls. The adaxial surface of the leaf-blades possesses papillae which aid the wax in preventing wetting.

The most active stomata, which occur on the sides of the furrows, show interesting auxiliary structures in the form of forked papillae on the subsidiary cells. These bend over the slit, forming a fringe which entangles an air-bubble when the leaves are submerged, and thus prevent the flooding of the air-spaces. The other stomata are of the normal grass type, but in some positions they are inert, owing to the absence of chlorophyll and the small osmotic content of the guard cells.

Numerous hydathodes of a hitherto undescribed type occur along definite tracts in the neighbourhood of large water-storing cells. These excrete large quantities of water and salts in solution. This exudation is due to some form of protoplasmic activity within the hydathode rather than to root pressure.

Hairs are abundant on the young vegetative organs, but most disappear very early. They persist on the glumes, where they may help in the opening and closing of the flower, and also in entangling air-bubbles when submerged.

There is a large development of air-passages in all organs of the plant. Their origin varies in different parts.

The two types of roots show differences in thickening and the degree of air-passage formation. A remarkable feature is the presence of solid portions in the deep penetrating roots, where numerous air-passages would seem more in keeping with accepted views.

In conclusion, we wish to express our thanks to Dr. O. Stapf and Mr. L. A. Boodle for helpful suggestions and criticism. We are also indebted to the Director of Kew Gardens for permission to work in the Jodrell Laboratory, where part of this work was completed.

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NOTES.

ON THE 'PROLIFEROUS' FORM OF THE SCAPE OF *PLANTAGO LANCEOLATA*.—In Masters' 'Teratology' there will be found a description, at pp. 108 et seq., of various forms of abnormal scapes in the genus *Plantago*. They are put into five groups, after Schlechtendal (Bot. Zeit., 1857, p. 873). Two of these groups are: the 'roseate' form of *Plantago media*—the garden rose-plantain; and the 'proliferous' of *Plantago lanceolata*. The other three need not concern us, but the suggestion is made that all five are built on the same plan, that is, of growth-increase in the various parts *already existing* in the spike. Masters, describing the 'proliferous' form of spike, says it consists of 'several spikes, some sessile, others stalked and pendent, the whole intermixed with leaves and disposed in a rose-like manner'.

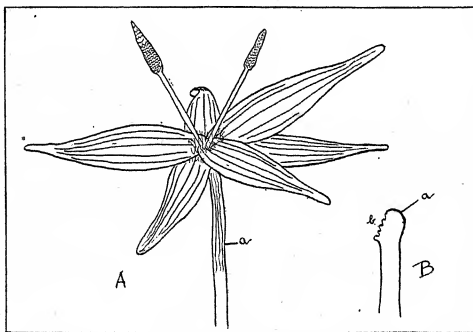


FIG. 1. First specimen of vegetative abnormality on the scape of *Plantago lanceolata*.

The 'proliferous' form is rather rare, but the writer has to record the finding of two specimens in two different localities near Dublin in one afternoon, viz. by the towpath of the Royal Canal at the Liffey Junction Station and near the top of Knockmaroon Hill.

First of all, as to the general features of the abnormalities. Fig. 1, A, is a representation of the first specimen. The large ribbed flower scape (*a*) is shown. It was surmounted by a plant—much more rosette-like than the parent—which consisted of seven well-developed leaves. Only six are drawn. The leaves were fairly large,

varying in length from $3\frac{1}{2}$ to 6 inches. There were also borne two small secondary flower scapes with good spikes which are also shown. The details of the apical growth were not followed, but the white hairs which clothe the leaf bases of the typical plant were well developed here. One point was clear, however, and is shown in Fig. 1, B. It represents the flower scape with the vegetative portion removed, and shows that its point of attachment (β) was lateral. The part (α) above this was withered and dry, as if some part previously growing above it had died.

The second specimen showed more. It was obviously younger—only five leaves being developed, and these being small. The rosette was somewhat asymmetrical. Fig. 2 shows this, and further that it was asymmetrical because it was carried as a lateral outgrowth from the lower end of a normal spike of which the withering head is still seen projecting on the left, being pushed aside by the developing rosette. The lower part of the spike—hidden in the figure—was still visible. Fig. 1 is but an older stage of this, in which the spike has withered and the vegetative growth has become practically symmetrical—its lateral origin being still indicated, as in Fig. 1, B.

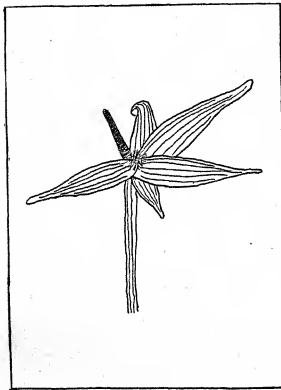


FIG. 2. Second specimen of abnormality on *Plantago lanceolata* (also diagrammatic).

The abnormality is apparently to be interpreted as the adventitious development of a vegetative bud in place of a flower-bud in the spike, comparable with similar developments in the flower heads of other plants, such as in the case of the *Pelargonium zonale* mentioned by H. de Vries (Jahrb. f. wiss. Bot., Bd. xxi).

The remarks it is wished to make are these. In the first place it is quite a different phenomenon from the development seen in *Plantago media* var. *bracteata*. This is a well-known constant garden variety of which there is a very good example in the Botanical Gardens here. In this form the bracts subtending the flowers of the spike grow vegetatively. They may only undergo this development

along part of the spike or throughout its whole length.

Passing on now to the second point, which relates to the anatomical construction of normal and abnormal scapes derived from specimen A. A transverse section of the normal scape shows a ribbed structure with development of sclerenchyma towards the periphery, and especially at the ribs. Within the sclerotic zone are complete vascular bundles of moderate dimensions, and in addition isolated strands of phloem are present.

The abnormal scape had a larger section, due at any rate in part to the increased dimensions of the cortical parenchyma. In addition to this, however, the

vascular bundles are larger than in the normal specimen, whilst the isolated phloem strands are replaced by small, complete vascular bundles. A final point of difference consists in the more complete development of the sclerenchyma zone in the abnormal specimen. It was better differentiated (more highly sclerized), and also consisted of more numerous cell-layers. The relative increase of this 'mechanical' tissue was greater than that of the 'vascular' elements.

These modifications the writer attributes to the joint requirements of a flower scape which has to sustain and carry water to an extensive vegetative development, such as that represented in Fig. 1— requirements which are both transpiratory and mechanical.

Some time ago, as a consequence of grafting buds on petioles of *Pelargonium zonale*, the writer found that when the buds grew into large plants the petioles developed secondary thickening, interfascicular cambium, periderm, and an indefinite extension of life (Proc. Roy. Dubl. Soc., vol. xiv, no. 33, 1915). In the paper cited he gave reasons for the contention that the secondary thickening in the grafted petioles was not entirely due to the stimulus of increased transpiration requirement (as claimed for similar phenomena by Winkler in Jahrb. f. wiss. Bot., Bd. xlv, 1907), but also largely as a response to some mechanical stimulus from the obviously increased stresses to which the petiole was subjected. This contention seems to be borne out by the circumstances described for the case of *Plantago lanceolata*.

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ON THE RELATION BETWEEN TRIGONOCARPUS AND GINKGO.—Miss Affourtit and Miss La Rivière, in their paper entitled 'On the Ribbing of Seeds of *Ginkgo*' (Ann. Bot., October, 1915), writing of the comparison of *Ginkgo* with *Trigonocarpus*, state (p. 594) that 'Since in *Ginkgo*, however, no valves occur—the stony coat lacking fissures at the plane of the ribs—and as vascular bundles are absent from the sarcotesta, those seeds cannot, as it seems to us, be compared with the seeds here described'. With regard to the first objection, it has been pointed out by the writer (Ann. Bot., January, 1914) that the *Trigonocarpales* show almost every transition with regard to the occurrence of commissural ribs. In *Polylophospermum* both the major and secondary ribs were commissured. In the genus *Trigonocarpus* itself the fissured character had entirely disappeared from the secondary ribs and was not uniformly exhibited by the major ones. Moreover, in the closely related *Stephanospermum* both ribs and commissures have entirely disappeared. The absence of fissures in the major ribs of *Ginkgo* is therefore merely a further stage in the evolutionary tendency exhibited by the genus *Trigonocarpus*, and it is significant that, as pointed out by Carothers (Bot. Gaz., 1907, p. 126), the integument of *Ginkgo* readily splits in the plane of the ribs.

The absence of sarcotestal bundles in *Ginkgo* can no more be taken as precluding affinity between the two groups than the presence of vascular strands in the integuments of some angiospermous ovules invalidates their comparison with ovules in which an integumental vascular system is lacking.

In view of the absence of sarcotestal bundles, the non-development of tertiary ribs calls for no explanation.

It is probable that the two vascular bundles (three in three-angled seeds) of the *Ginkgo* ovule correspond to the nucellar supply of *Trigonocarpus*.

This is indicated by the facts that they pass up close to the plane of fusion between nucellus and integument, and that, though serving as the vascular supply for both structures, the bundles end at the level at which the nucellus becomes free.

That the number and position of the strands should correspond to that of the ribs of the integument is not surprising, seeing that the angling of the nucellus shows a like correspondence as to number and position. Moreover, in *Trigonocarpus shorensis* it was found that the number of bundles in the nucellar system was a multiple of three, corresponding with the trimerous character of the integument.

In the taxonomically more important features of general organization the ovules of Ginkgoales, Cycadales, and Trigonocarpaceae exhibit a uniformity of construction difficult to explain except on the basis of affinity. The morphological and anatomical characters of these groups, whilst emphasizing the closer relation between the Trigonocarpaceae and Cycadales, lend further support to the hypothesis of the affinity of all three. On such a view, the large proportion of *Ginkgo* ovules with three ribs recorded by Affourtit and La Rivière has an added significance.

E. J. SALISBURY.

EAST LONDON COLLEGE,
January, 1916.

STIGEOSPORIUM MARATTIACEARUM, gen. et sp. nov.—In the course of a comparative investigation of the anatomy of the Marattiaceae, a new mycorrhizal fungus, for which the generic name *Stigeosporium* is proposed, was noticed by the writer in roots of various genera of the above-mentioned group of Ferns.

Special interest attaches to this mycorrhizal fungus inasmuch as it produces *under natural conditions distinct reproductive bodies* (other than 'vesicles') *within the tissues of the host-root*.

The more important characters of this fungus are included in the following brief diagnosis:

DIAGNOSIS.

Stigeosporium, gen. nov.

Mycellium ramosum ex hyphis inter- et intra-cellularibus continuis rarissimè septatis constans; haustoriis numerosis, extremitatibus in ramulis radiatis valde dissectis; sporis perdurantibus solitariis plerumque globosis raro subglobosis et cet., membrana crassissima irregulariter intense colorata.

Differt a *Phytophthora*, cui arcte affine, habito symbiotico (igitur conidia normalia non formantur).

Sp. unica.

Stigeosporium marattiacearum, sp. nov.

Hyphae initio hyalinae demum flavo-brunneae vacuatae 1–12 μ crassae; sporis aut intercalaribus aut terminalibus plerumque globosis 32–45 μ diametro, raro subglobosis vel ovoides vel pyriformibus; exosporio hyalino levi; mesosporio crassissimo 6 μ crassitudine minute punctulato flavo irregulariter intense colorato; endosporio tenui.

Hab. In radicibus subterraneis specierum variorum generum (*Angiopteris*, *Archangiopteris*, *Kaulfussia*, *Marattia*) Marattiacearum symbioticum.

Distrib. Asia orientalis, Zeylan, Australasia.

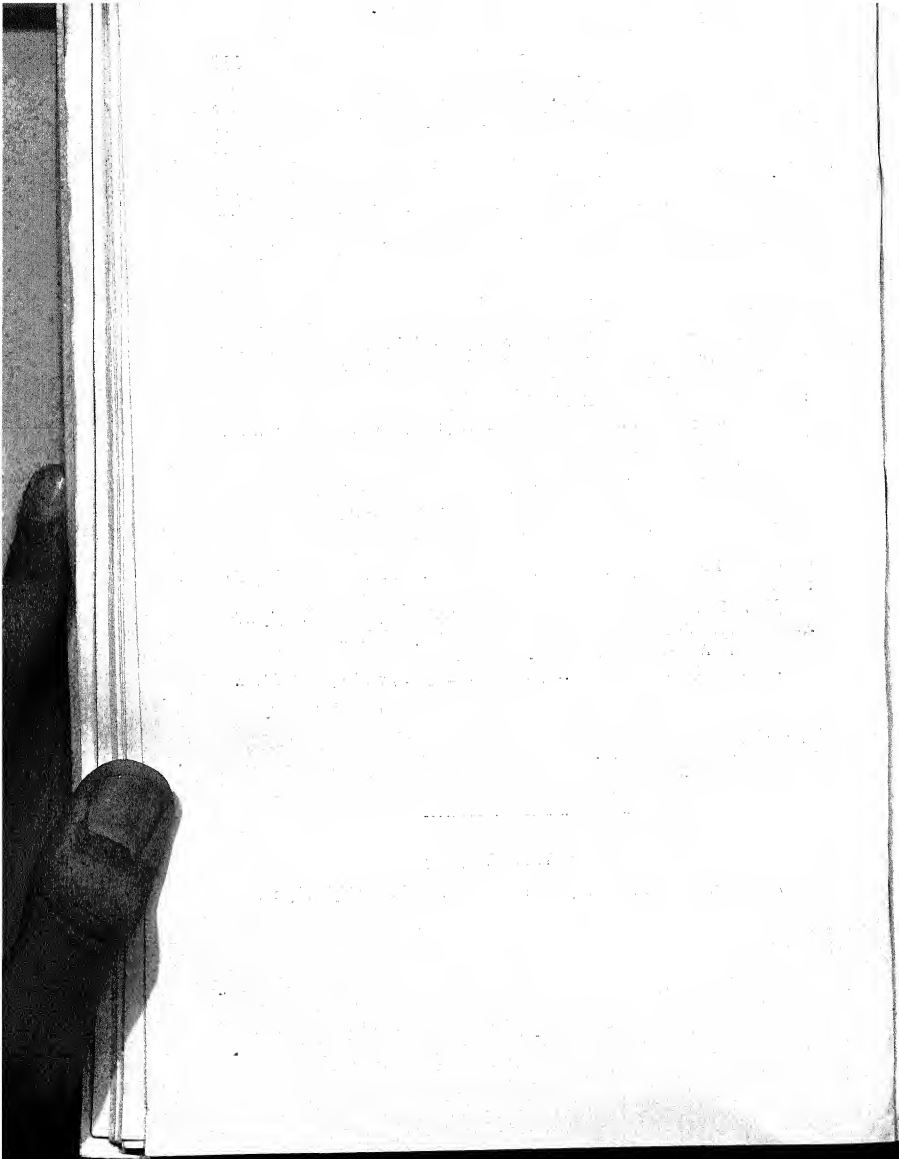
A full description of this interesting fungus, with figures, will be published later.

CYRIL WEST.

ROYAL COLLEGE OF SCIENCE,
SOUTH KENSINGTON, S.W.
1915.

ANN. BOT., Vol. XXVI, p. 205.

The dimensions given on this page in terms of μ should be multiplied by 10.



Alfred Stanley Marsh.

ALFRED STANLEY MARSH was born at Crewkerne on February 1, 1892. He died on January 5, 1916, being shot through the heart by a German sniper, who had found a weak spot in the parapet of the trench. Death was instantaneous. One who ran to his aid was killed in the same way.

Marsh was educated from 1903 to 1909 at Sexey's School, Bruton, and here his taste for Natural History received encouragement. He obtained a First Class in both the Junior and Senior Oxford Local Examinations, and in both he gained distinction in Botany (among other subjects). In 1908, when only sixteen years of age, he gained the County Scholarship and an Exhibition and Subsizarship at Trinity College, Cambridge; but he did not enter Cambridge University until 1909, when he also won the Soley Scholarship. He surmounted the obstacle of the Latin and Greek tests in the Little-go, being placed in the third class, and wiring to his Head master, characteristically—'Sorry I have passed one class higher than I intended.'

As an undergraduate at Trinity his record was a distinguished one. In 1911 he obtained a First Class in the Natural Sciences Tripos, Part I. He then specialized in Botany, and in 1913 he was placed in the First Class in Part II of the same Tripos. He was thereupon offered the Frank Smart Studentship in the gift of Gonville and Caius College, accepted it, and migrated from Trinity to Caius. This Studentship afforded him the opportunity of doing research work in Botany, of which he availed himself with energy and enthusiasm. He acted as a demonstrator in Professor Seward's classes in Elementary Botany, and on Dr. Moss's field excursions, his keenness and good humour endearing him to the students.

Marsh's published work consists of four papers which appeared in 1914-15:

(1) 'Notes on the Anatomy of *Stangeria paradoxa*.' New Phyt., xlii, pp. 18-30. 1914. Marsh demonstrated an interesting mode of vascular supply of the leaves in this Cycad, and followed the behaviour of the centripetal and centrifugal xylem strands of the leaf throughout its whole length. His conclusion was that 'a close relationship can be argued between the modern Cycadales and the fossil Cycadofilices'.

(2) 'The History of the Occurrence of *Azolla* in the British Isles and in Europe generally.' Proc. Cambridge Phil. Soc., xvii, pp. 383-6. 1914. This paper gives an account of the occurrence of *A. caroliniana*

[Annals of Botany, Vol. XXX. No. CXVIII. April, 1916.]

and *A. filiculoides* in Britain, and is a valuable basis for the study of these two interesting and rapidly spreading aliens.

(3) 'The Anatomy of some Xerophilous Species of *Cheilanthes* and *Pellaea*.' Ann. Bot., xxviii, pp. 671-84. 1914. In this paper Marsh gave a comparative account of the stelar anatomy of several closely related Ferns; and in the case of the peculiar vascular structure of the petioles he showed that they could all be derived from Sinnott's primitive type with three endarch protoxylems.

(4) 'The Maritime Ecology of Holme-next-the-Sea, Norfolk.' Journ. of Ecology, iii, pp. 65-92. 1915. This is the report of a valuable piece of work undertaken by a group of young Cambridge men, altogether ten in number. A short account of the work was given in 1913 before the British Association by P. H. Allen ('by whose untimely death', Marsh writes, 'we lost a dear friend and a splendid fellow worker'). Marsh was the chosen spokesman of this group of (in more senses than one) synecologists, and his paper is an admirable example of vegetational survey work and a demonstration of the possibilities of co-operation in ecological research.

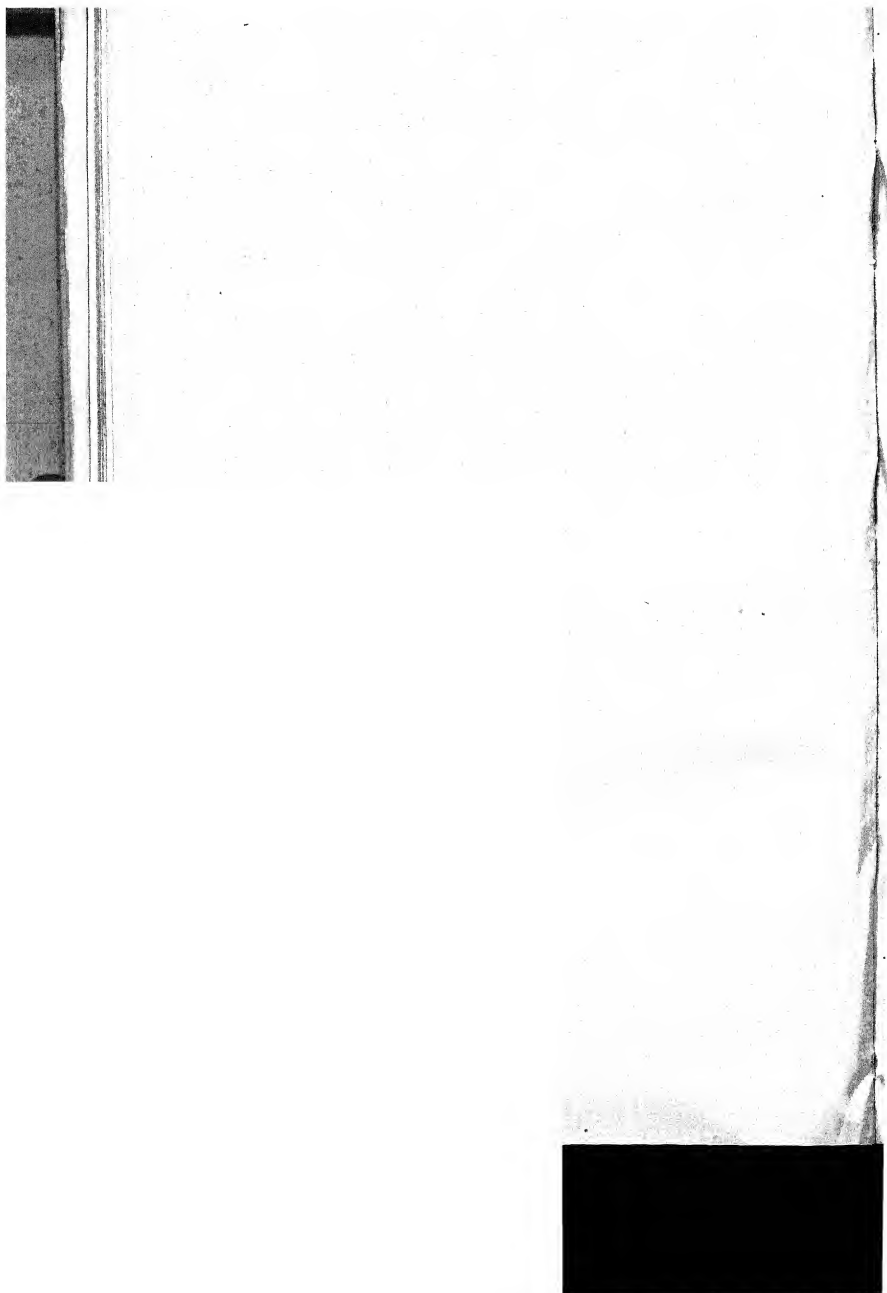
Marsh took a commission in November, 1914, in the 8th (Service) Battalion, Somerset Light Infantry. He was made First-Lieutenant in April, 1915, and got his Captaincy in August, being then only twenty-three years of age. Throwing himself whole-heartedly into his new duties he quickly won popularity with his men and the affection of his fellow officers. Letters from the Front, from officers and men alike, testify to the deep sorrow which his death inspired even among those to whom bereavements are of the texture of daily life.

While devoting himself entirely to his military duties he longed for the time when he should be able again to take up the botanical work that he loved. His plans for the future included a continuance of the synecological work at Holme, a series of experiments on the relations of closely allied species to their different habitats, and a share (with his friend R. C. McLean) in an investigation of flower structure in the Ranales. His botanical interests were broad, and to every discussion he brought fresh and suggestive ideas and a power of seeing the distant implications of hypotheses. His earliest botanical enthusiasm was floristic, and he astonished one by his uncanny ability to state accurately the 'census-number' of every British flowering plant. His Cambridge training opened his eyes to the manifold avenues of research which Botany presents; and though he had not yet settled down to any one definite line of work, it would probably have been some branches of Phylogenetic Anatomy and Ecology which would have claimed him. By his death botanical science is the poorer for the loss of a keen vision, an agile imagination, and an enthusiastic capacity for work.

His personality was of the frankest, merriest and most good-humoured. He radiated gaiety and good fellowship. Always ready for a jest, his quaint wit enlivened many a conversation. His sense of the odd possibilities of words led him into the paths of humorous verse and prose. He was editor of, and responsible for a great part of the matter in the latest number of that celebrated periodical 'The Tea Phytologist'. His cheerful goodwill and appreciative outlook upon life made his presence the best possible antidote to depression. All who knew him sincerely mourn the loss of his bright and stimulating personality. To his many devoted friends his death brought bitter grief for the passing of one whose comradeship is among the happiest memories of their lives.

R. H. C.

CAMBRIDGE,
March, 1916.



Experimental Investigations on the Genus *Drimys*.

BY

PROFESSOR EDWARD C. JEFFREY,

Of Harvard University,

AND

RUTH D. COLE,

Of Radcliffe College.

With Plate VII.

EXPERIMENTAL investigation has come prominently into the foreground in many lines of botanical work in recent years. In no case, perhaps, is its value so clear as in the case of the Conifers, which present the great advantage of a long anatomical history displayed in the strata, by means of which experimental results may be controlled. An interesting general result which has been derived from united palaeobotanical and experimental study in the case of the Conifers is that the more simply organized subtribes of the group are derived from ancestors with more complex anatomical structures. One of us pointed out a number of years ago,^{1, 2} that in the genus *Sequoia* and in those genera of the Abietineae without normal resin canals in the secondary wood (*Abies*, *Tsuga*, *Pseudolarix*, and *Cedrus*), resin canals reappear as a consequence of injury. This reappearance of secretory canals as a phenomenon of injury is particularly significant because these structures are found to a large degree normally in the more conservative regions of the genera named. Later investigations have shown further that the rays of certain Conifers may be modified experimentally in an interesting way. First, in the genus *Cunninghamia*³ it was demonstrated that a frequent result of injuries was the appearance of marginal ray tracheides, such as are characteristic of the pine-like

¹ Jeffrey, E. C.: The Comparative Anatomy of the Coniferales. I. The genus *Sequoia*. Mem. Boston Soc. Hist., vol. v, 1903, pp. 441-59, Pls. 68-71.

² Jeffrey, E. C.: The Comparative Anatomy of the Coniferales. II. The Abietineae. Mem. Boston Soc. Nat. Hist., vol. vi, 1904, pp. 1-37, Pls. 1-7.

³ Jeffrey, E. C.: Traumatic Ray Tracheides in *Cunninghamia sinensis*. Ann. Bot., vol. xxii, 1908, pp. 593-602, Pl. XXXI.

Abietineae but are not normally present in the rays of this genus. Later, Miss Holden¹ made clear that the possibility of the recall of marginal tracheides was widespread in both the Taxodineae and Cupressineae. Professor W. P. Thompson² has demonstrated a similar situation in the genus *Abies* among the higher Abietineae, where, as a consequence of injury and especially in the root, ray tracheides make their appearance. Recently³ it has been shown that in the existing araucarian Conifers the parenchymatous elements found as a normal feature of organization of the wood of many Mesozoic Araucarioxyla may be recalled by experimental means. This situation is none the less significant because the presence of wood parenchyma is a normal feature of organization of the conservative parts of the living representatives of the Araucarineae.

It will be clear from the summary statement made in the last paragraph that experimental methods have been of great value in the study of the evolutionary history of the coniferous Gymnosperms. In the case of the Angiosperms the fossil record is at the present time extremely incomplete so far as structural remains are concerned; and the study of existing forms by experimental procedure is as a consequence of even greater importance relatively than it is in the Conifers. In the present communication it is proposed to show the value of the study of abnormal material in the case of the non-vascular magnoliaceous genus *Drimys*. The absence of vessels in this interesting genus of the Southern Hemisphere was noticed in the early forties of the last century by Goepfert (Goepfert, *Linnaea*, 16, 1842). This condition is of particular interest in view of the speculations in regard to the origin of the Angiosperms, which in the case of certain investigators have been made to centre around the Magnoliaceae. The floral organization of the Magnoliaceae and related families has been the main point of argument in this connexion. Obviously it is well to consider possible evidence of the primitive character of the group from anatomical characteristics. Clearly the absence of vessels, if an original condition in the Magnoliaceae, would furnish a strong indication in this direction, were the gymnospermous forms hypothetically antecedent to the Angiosperms themselves non-vascular. If the Gnetales are ancestral to the Dicotyledons, as has been suggested by Arber and Parkin⁴ as well as other investigators, evidently the absence of vessels in any angiospermous

¹ Holden, Ruth: Ray Tracheides in the Coniferales. *Bot. Gaz.*, vol. lv, No. 1, Jan. 1913, Pls. I and II.

² Thompson, W. P.: Ray Tracheides in *Abies*. *Bot. Gaz.*, vol. liii, 1912, pp. 331-8, Pls. XXIV, XXV.

³ Jeffrey, E. C.: The History, Comparative Anatomy, and Evolution of the Araucarioxylon Type. Part I. *Proceedings of the American Academy of Arts and Sciences*. Vol. xlviii, No. 13, Nov. 1912.

⁴ Arber, E. A. Newell, and Parkin, John: On the Origin of Angiosperms. *Journ. Linn. Soc., London, Bot.*, vol. xxxviii, 1907, pp. 29-80.

group cannot with any degree of probability be regarded as a primitive feature.

The material used in the present investigation was collected at the request of the senior author by travelling Fellows of Harvard University in New Zealand and in Java, and care was taken to secure seedlings as well as parts of mature plants. Wounded conditions were particularly sought for on account of the proved value of wound reactions in the case of both Conifers and Dicotyledons. Abundant material of *Drimys colorata* and a moderate amount of *D. axillaris* were available from this source. We are indebted to the Director of the Royal Gardens, Kew, England, for small twigs of *D. Winteri* and *D. aromatica*. Through his kindness we have likewise been enabled to study the organization of the interesting genera *Trochodendron* and *Tetracentron*, which are also devoid of typical vessels.

The wood of *Drimys*, apart from the absence of vessels, strongly resembles in general appearance that of one of our northern oaks, for it is characterized by conspicuously large rays in striking contrast to others a single row of cells in breadth. Fig. 1, Pl. VII, shows the general organization of the wood of *D. colorata* as seen in transverse section. To the right and left are indications of the broad rays of the oak type. The mass of wood in the centre is radially traversed by narrow and uniseriate rays. The annual rings are somewhat indistinct, a result of the mild climatic conditions under which the plant has flourished. They are most clearly marked by a terminal zone of parenchyma. Fig. 2, Pl. VII, reproduces the tangential aspect of the wood in *D. colorata* under a moderate degree of magnification. Laterally are the large rays as in the preceding figure, and in the centre lies the mass of wood penetrated by the linear or uniseriate rays. Wood parenchyma is not seen in the field of view. Fig. 3, Pl. VII, shows a part of the wood of *D. colorata*, somewhat highly magnified. The narrow rays alone are evident, and the fact that they are uniseriate is now quite apparent. The mass of the wood is composed of tracheides which are thick-walled and angular in shape. Wood parenchyma appears tangentially as thin-walled elements which look black in the photograph.

The characters of the longitudinal elements or tracheides are of greatest interest in the present connexion. Fig. 4, Pl. VII, represents a rather high magnification of a radial view of the wood in *D. colorata*. To the right may be seen parenchymatous cells, the remainder of the field showing only tracheary elements. The pits in the latter are in a single row and do not usually become flattened by mutual contact, thus scarcely justifying the comparison with the wood of the araucarian Conifers which has often been made. Fig. 5, Pl. VII, supplies a view of the wood of *D. axillaris* under a similar magnification. Parenchymatous elements are

in this case likewise present on the right, while the illustration as a whole shows only parts of tracheides. The pits are appreciably smaller than are those of *D. colorata* in the preceding figure. Fig. 6, Pl. VII, shows a view of the root wood of *D. colorata*. The pits are now in two rows, in contrast to the single series characteristically present in the woody structures of the stem. The increase in number of pits appears to be a usual feature of organization of the wood of the root both in Dicotyledons and Conifers. The pores of the tracheides in the figure alternate in the araucarian manner, but are not deformed by approximation and mutual pressure as in the subtribe of Conifers indicated.

The most interesting conditions which have been observed in the present connexion have been found in the case of the root wood, when injured. Two large adult roots and the roots of a number of seedlings of *D. colorata* showed the presence of healed wounds. For comparison with these was available a large amount of normal material. It should be stated that the results recorded here depend on the examination of a considerable number of preparations, and have to that extent a general validity. Fig. 7, Pl. VII, reproduces part of a longitudinal radial section of a wounded root in the vicinity of the injury. To the left in the figure appear tracheides filled with a dark gummy material, which is one of the consequences of injury. A curious phenomenon is seen in some of the tracheides, most strikingly visible in the fourth element from the right. Here, instead of the rows of separate rounded pits, which are characteristic of the normal structure of the root, we find elongated scalariform pits, such as are often present in vessels of the Magnoliaceae and other dicotyledonous families. To the left of the element under discussion is one in which the scalariform pitting is less pronounced, and in the next tracheide to the right of the first described it can scarcely be observed. Fig. 8, Pl. VII, shows another field of view from a different root (likewise wounded). Crowded pitting occurs in the tracheides on the right and left of the figure. To the left of the centre in the figure lies a tracheide in which scalariform pitting is a marked feature. A careful inspection of the pits in the upper and lower regions of this element makes it clear that the scalariform pits are the result of the fusion of the rounded pores, which are a feature of the organization of the normal tracheides of the wood of the root. Two or three rows of parenchyma can be made out in the illustration. Fig. 9, Pl. VII, furnishes us with still another view of injured wood in the root of *D. colorata*. Here may be observed a great variety of conditions in regard to the fusion of pits in the tracheides. In an element to the left of the centre the condition of fusion is very marked, and the scalariform pores resulting from it are as a consequence much elongated. Farther to the right and left all stages of fusion may be made out in the case of the small round pits, which are a normal type of pore in the elements of the root. An examination of

a considerable amount of material makes it clear to us that, so far as the root of *D. colorata* is concerned, the appearance of scalariform pitting is the usual result of injury in a smaller or larger number of the tracheides. Similar phenomena were found to hold for seedlings as well as the root of the adult.

It is natural to compare the conditions resulting from injury in the stem of *D. colorata* with those found in the root. A much more limited amount of material was at our disposal in the case of this organ. A large number of sections have been prepared, however, both of the adult and seedling wounded stem. In no instance was there any indication of the fusions of pits such as are found quite generally in the lateral walls of the vessels of the vascular Magnoliaceae, as well as other dicotyledonous families ordinarily regarded as holding a higher systematic position. It seems natural to explain the difference of behaviour of these two organs in the case of injuries, so far as they may not be due to the limited amount of material of the injured stem, to the greater conservatism of the root. The significance of this statement will become apparent at a later stage.

As a preliminary to the interpretation of the results of injury in the case of *Drimys*, it is necessary to devote some attention to the general features of organization of the vessel in the Magnoliaceae (including the Trochodendraceae) as a whole. The monotypic genus *Liriodendron* will serve to point the present remarks, although it is well to state that a large number of other genera have likewise been examined in the present connexion. Fig. 10, Pl. VII, shows the organization of one of the vessels in the root of *Liriodendron tulipifera*. The vessel is flanked on either side by other elements of the wood, both fibrous and parenchymatous. The trachea or vessel is of most importance from the standpoint of the interpretation of the traumatic phenomena of *Drimys*. In the upper and lower regions of the wall may be seen rows of opposite pits, which are clearly bordered and serve to bring about lateral relations with an adjoining vessel. Starting from the middle of the vascular element and descending may be seen a region of actual perforation, affording an unimpeded communication with another below the plane of section. The apertures in this case are the result of the loss of borders and likewise the partial fusion of the pits, which characterize the lateral walls of the vessel. The correctness of this interpretation may be easily inferred from an inspection of the figure. For comparison with Fig. 10, Pl. VII, another vessel from *Liriodendron* of somewhat different type is presented in Fig. 11, Pl. VII. Here the lateral pits are for the most part elongated and belong to the type known as scalariform. In the region below the middle the vessel is perforated in this instance, as in the other case, so that the openings have resulted from the lateral pits having lost their membranes as well as their borders.

The fusion of pits observed in the foregoing figure is not here apparent, since it has already taken place in the lateral walls.

It is clear from the observations recorded in the preceding paragraph that the vessels of *Liriodendron* are distinguished from the tracheides by their larger calibre, the numerous pits of the lateral walls, either opposite or fused to constitute scalariform pores; and finally by the scalariform perforations, bringing about open communication between vessel and vessel. These perforations are obviously the result of modification of the opposite rows of lateral pits or of the scalariform fusions of these. It thus becomes evident that the vessel in types like *Liriodendron* is not far removed from the tracheide in its organization, although it possesses the distinctive features of a vessel. In some instances the perforations may be absent, but the essentially vascular character of the element may then be inferred from the numerous opposite or fused (scalariform) pits of its lateral walls, as well as from its characteristically large calibre. There is an interesting resemblance between the vessels of *Liriodendron*, in fact, and those found in certain Ferns and other Vascular Cryptogams. In *Pteris aquilina*, for example, vessels take their origin from the disappearance of the borders and membranes of certain of the scalariform pits of the wall. It is important to note, however, that in *Pteris* and similar forms the scalariform pitting is a primitive feature of organization, while in the Dicotyledons it has been derived secondarily by the fusion of opposite rows of pits in the lateral walls of the vascular elements.

The considerations put forward in the two preceding paragraphs bring us to the discussion of the scalariform elements found in proximity to the protoxylem in *Drimys* and in an indefinitely wider region in *Trochodendron* and *Tetracentron*. Those scalariform elements lying farther away from the primary region of the wood are of significance in the genus *Drimys*. Here one finds a marked tendency to scalariform pitting in the terminal regions of the tracheary elements, after it has been superseded in the rest of the walls by typical round bordered pits. In the case of *Drimys* we are left in doubt by reason of the proximity of the elements to the scalariform tracheides of the primary wood. In *Trochodendron* and *Tetracentron*, however, this ambiguity does not occur, since the vessel-like tracheides are found in regions far outside the primary wood and consequently cannot reasonably be interpreted as persistent scalariform elements of the first-formed wood.

The conditions resulting from wounding in the case of *Drimys*, nevertheless, seem to throw more light on the subject under discussion than is afforded by the study of the first annual ring, the leaf, and the root; all regions which we have been led as a consequence of the investigations on gymnospermous anatomy in recent years to regard as the seats of ancestral characters. By reason of the possibility of the confusion of

degenerate vessels with the similarly organized scalariform elements of the primary wood the question of interpretation becomes difficult. The situation, therefore, may be compared somewhat accurately with that in *Sequoia sempervirens*. In this species resin canals are formed as a result of injury, and do not, as in the allied species *S. gigantea*, occur in the primitive regions—first annual ring, leaf trace, and cone axis and its scales. Traumatism in the redwood (*S. sempervirens*) supplies, in fact, the only evidence as to its former possession of resin canals, while in the big tree (*S. gigantea*) traumatic evidence is reinforced by the conditions found in the conservative organs. The situation in *Drimys* may also be compared quite accurately with the wood phenomena presented by the rays of the Taxodineae and Cupressineae in general as described by Miss Holden¹ and one of us.² Here the return of ray tracheides has been observed only as the result of injury, and is not found normally in any of the regions recognized as conservative.

But if it be granted that the vessel-like structures which occur traumatically in the injured root are distinct evidence of the former presence of vessels in *Drimys*, we have still certain difficulties to consider. First, there arises the question whether the structures under discussion are in reality to be regarded as degenerate vessels in reversionary return. Secondly, there is the equally important problem as to whether it is inherently probable that any group or genus of Angiosperms can primitively have possessed vessels and have subsequently lost them.

Taking the question of interpretation first, we may ask if the peculiar scalariform elements occurring in the root of *Drimys* after injury are in reality to be interpreted as of the nature of vessels. They are certainly not to be considered as tracheides, since the sculpture of their walls is quite unlike that found in tracheides in general, and entirely resembles that observed as characteristic of vessels in the Magnoliaceae and other families. The only difference between the structures in question and typical vessels is the absence of actual perforations. This, however, is not a serious objection. In the Cactaceae and Crassulaceae among the Dicotyledons are found vessels which by the loss of their terminal pores have ceased to be technically of the character of vessels. An examination of the genus *Opuntia* among the Cactaceae by Miss Bliss, working in this laboratory, has made it clear that what are occluded elements of a vessel-like nature in the later wood are patent vessels in the region of the pith. In certain of the Magnoliaceae, where the vessels have not only scalariform perforations but also scalariform pits on their lateral walls, we have merely to imagine

¹ Holden, Ruth: Ray Tracheides in the Coniferales. Bot. Gaz., vol. lv, No. 1, Jan. 1913, Pls. I and II.

² Jeffrey, E. C.: Traumatic Ray Tracheides in *Cunninghamia sinensis*. Annals of Botany, vol. xxii, 1908, pp. 593-602, Pl. XXXI.

the scalariform perforations obliterated by the reduction in calibre and development to realize structures of the same nature as those occurring traumatically in *Drimys*. This interpretation, moreover, gains force from the fact that in *Liriodendron* such degenerate vessels are of normal, although rare, occurrence. Further, the general phenomena of traumatism lead us to expect, more often than not, the recall of ancestral characters in an abnormal form. This is, for example, pre-eminently true of the traumatic resin canals and traumatic ray tracheides of the Conifers. A final argument for the interpretation of the curious elements appearing in the root of *Drimys* as a consequence of injury as reversionary indications of the former presence of vessels in the genus is that such an hypothesis explains the fact satisfactorily. If the opposition and fusion of pits in the tracheides of the root in *Drimys* were only a meaningless vagary, we should expect to find parallel conditions in the injured woods of Conifers. Such have never been described.

We may now pass to the question of the inherent probability of the suppression of vessels in angiospermous woods. The evidence appears to be overwhelmingly in favour of such a possibility. Fig. 12, Pl. VII, illustrates part of the woody cylinder of *Alnus japonica*. In the centre there is a broad radial zone of the wood devoid of vessels, and laterally several similar non-vascular stripes of less diameter may be seen. The broad central band of vessel-less wood corresponds in position to the leaf trace. Such conditions are found in a number of cases in woody Dicotyledons, and are of very wide occurrence among the herbaceous representatives of the group. It has been shown by investigations carried out by students of this laboratory that the evascularization of wood is a phenomenon commonly related to the transformation of regions of the woody cylinder into parenchyma. In the case of *Alnus* figured above, it is quite easy to observe the gradual blotting out of the typical vascular organization as one progresses from the region of the leaf gap outwards. Since there is absolutely no question that vessels may degenerate in the Dicotyledons locally, there seems to be no difficulty in regarding as possible the occurrence of this phenomenon as a general feature of organization, particularly as this situation is actually realized in certain Cactaceae and Crassulaceae, as indicated above. Further, if the Gnetales or similar forms are ancestral to or cognate with the Angiosperms, the possession of vessels is clearly a primitive characteristic of the higher seed plants known as Angiosperms.

In conclusion we may attempt to picture to ourselves the type of wood structure from which *Drimys* has been derived. As has been pointed out in the beginning, the general organization of the wood, apart from the absence of vessels, is that of one of our northern oaks. There is good reason, both on account of its early abundant occurrence as a fossil and likewise on comparative anatomical grounds, to regard the oak as a relatively primitive

dicotyledonous type. If the resemblance to oak wood in general organization is of value, it is clear that *Drimys* has come from a comparatively primitive Ranalian type. Although the general structure of the woody tissues of the genus under discussion is primitive, the absence of vessels cannot apparently, in view of the present investigation, be so regarded. It seems quite clear that the ancestors of *Drimys* possessed vessels, and that these were of a type characterized by lateral scalariform pits and probably by scalariform perforations. The absence of perforations is merely a technical distinction between tracheides and vessels in the case of the Dicotyledon, and has no decisive evolutionary significance. No discussion of the evolutionary history in the case of vascular structures is complete unless the lateral as well as the end walls of the vessels are taken into consideration.

SUMMARY.

1. The roots in *Drimys*, in particular *D. colorata*, as a consequence of injury develop peculiar tracheary structures.
2. The structures in question are regarded as the abortive and reversionary return of vessels because of the resemblance of the sculpture of their lateral walls to that found in the vessels of the Magnoliaceae.
3. These traumatically induced elements are characterized by the opposition and fusion of rows of pits, and in this respect are clearly distinct from ordinary tracheides. They, however, lack the perforations of normal vessels.
4. In spite of the absence of perforations, they are apparently to be interpreted as a clear indication of the former presence of vessels in *Drimys* and similar forms among the Magnoliaceae.
5. *Drimys* is a representative of the Magnoliaceae primitive in position, as evidenced by its ray structures and the character of its traumatically recalled vessel-like elements.

DESCRIPTION OF PLATE VII.

Illustrating Professor Jeffrey and Miss Cole's paper on *Drimys*.

PLATE VII.

- Fig. 1. Transverse section of wood of *Drimys colorata*, showing absence of vessels and broad and uniseriate rays. $\times 40$.
- Fig. 2. Longitudinal tangential section of the wood of *D. colorata*. $\times 50$.
- Fig. 3. Transverse section of wood of *D. colorata*. $\times 125$.
- Fig. 4. Longitudinal radial section of wood of stem of *D. colorata*. $\times 125$.
- Fig. 5. Longitudinal radial section of wood of stem of *D. axillaris*. $\times 125$.
- Fig. 6. Longitudinal radial section of root wood of *D. colorata*. $\times 125$.

Fig. 7. Longitudinal radial section of wood of injured root of *D. colorata*. To the left tracheides occluded with gummy matter resulting from injury. To the right more or less vessel-like tracheary elements. $\times 75$.

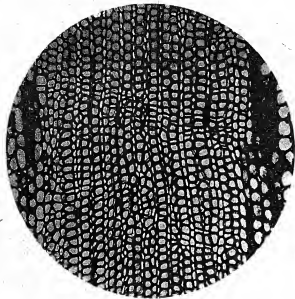
Fig. 8. Another longitudinal radial section of injured wood of root of *D. colorata*, showing wood parenchyma, tracheides, and one clearly vessel-like element. $\times 125$.

Fig. 9. Still another longitudinal radial section of the same, showing a variety of vessel-like structures formed as a consequence of injury. $\times 125$.

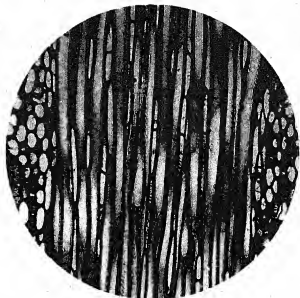
Fig. 10. Longitudinal radial section of the wood of the root of *Liriodendron tulipifera*. In the centre is seen a vessel of rudimentary type indicating clearly the origin of perforations from modified pits. $\times 75$.

Fig. 11. Radial section of root of *L. tulipifera*, showing vessel in the centre. The lateral pits of the vessel are scalariform, and the perforations originate from the loss of border and membrane in certain of these. $\times 125$.

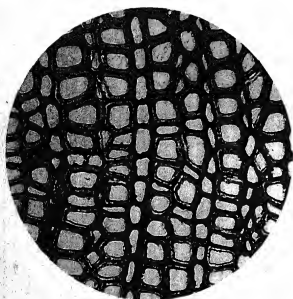
Fig. 12. Transverse section of portion of a three-year-old twig of *Alnus japonica*, showing the disappearance of vessels in the segment of the woody cylinder corresponding to a leaf trace. $\times 20$.



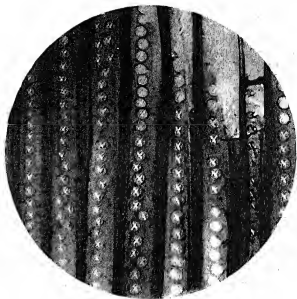
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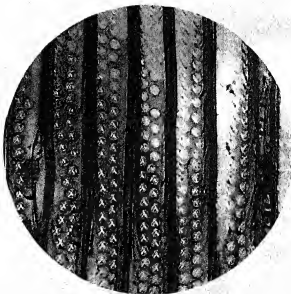
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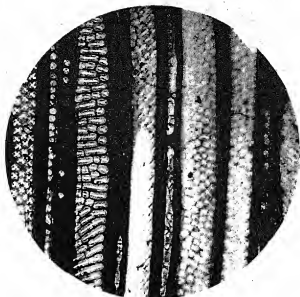


6.

JEFFREY & COLE-DRIMYS.



7.



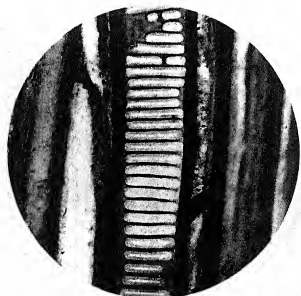
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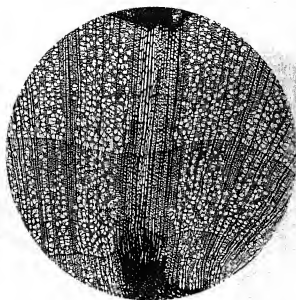
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12.

Huth coll.



On *Carteria* Fritschii, sp. nov.

BY

H. TAKEDA, D.I.C.

With ten Figures in the Text.

IN a paper on *Scourfieldia cordiformis*, published in the current volume of the Annals of Botany, reference is made to a species of *Carteria* which swims usually forwards but occasionally backwards.¹ Since this organism has proved to be a new species of that genus, a short account of its characteristics, together with a Latin diagnosis, illustrated by some careful drawings, will be given below.

The organism occurred abundantly in the same material as that in which *Scourfieldia cordiformis* was found, and which had been collected by Professor F. E. Fritsch at Keston, Kent, in May, 1915, and kept as a laboratory culture for over six months. The outstanding features of the organism are, firstly, that the outer firmer part of the cell-wall (i.e. the structure usually designated as 'cell-wall' or 'cell-membrane') is thicker than in any other described species of the same genus; secondly, that the inner gelatinous part of the cell-wall (i.e. that part of the cell-wall which lies between the outer firmer part and the protoplast, usually referred to as 'space') is frequently developed to a marked degree and very often unevenly, so that the protoplast does not always conform to the outline of the cell.

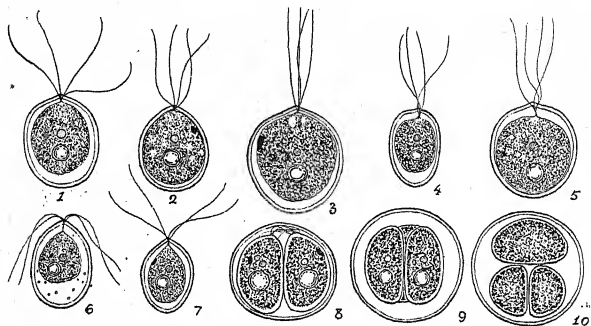
The organism varies in shape to some extent, being ovoid to nearly spherical, and in some cases obovoid-ellipsoid. When viewed from the end the organism usually appears to be circular, but occasionally broadly elliptical, the cell being slightly compressed from the side. At the anterior end of the cell there is no wart-like papilla, such as is seen in *C. obtusa*, Dill, but the cell-wall forms a slight obtuse angle without any conspicuous thickening. The outer firmer part of the cell-wall reaches a thickness of about $\frac{3}{4}\mu$, while the inner gelatinous part is often very well developed, particularly at the posterior end and sometimes at the anterior end of the cell. The gelatinous part of the cell-wall occasionally contains some colourless granular substance, the nature of which has not been determined (Fig. 6).

¹ Ann. Bot., vol. xxx, No. cxvii, January, 1916, p. 157.

[Annals of Botany, Vol. XXX. No. CXIX. July, 1916.]

The four flagella which are attached to the very small colourless beak at the anterior end of the protoplast as a rule slightly exceed the length of the cell. So far as has been ascertained, the flagella emerge through the cell-wall in four different directions, practically equidistant from one another.

There is a single urceolate chloroplast which is granular and occupies practically the whole of the protoplast. Enclosed within the central hollow of the chloroplast there is a mass of colourless protoplasm, at the bottom of which, and slightly nearer the anterior end than the centre of the cell, a small nucleus is lodged. It appears that from this central mass



Carteria Fritschii, Tak., sp. nov. $\times 1,000$. Figs. 1-7. Zoogonidia. Fig. 8. Mother-cell with two daughter-cells inside. Note the daughter-cells are provided with flagella. Fig. 9. Mother-cell with three daughter-cells. Fig. 10. Ditto, an end-view.

of protoplasm a number of processes radiate towards the periphery of the cell, penetrating through the chloroplast and finally reaching the thin peripheral layer of protoplasm. These protoplasmatic rays are frequently quite conspicuous, particularly at the point where they join with the peripheral layer of protoplasm (cf. Figs. 2, 5). A pyrenoid, which is usually globular or sometimes more or less angular, and as a rule quite conspicuous, is present near the posterior end of the chloroplast. It is situated usually in the axis of the cell, or it is occasionally excentric (Figs. 6, 7). There is a small yet conspicuous stigma (pigment-spot), oval in shape and somewhat anterior in position (Figs. 2, 3, 7, 8). Two contractile vacuoles, which pulsate alternately, can be seen just below the protoplasmatic beak (Fig. 3).

Two to four daughter-cells are produced as the result of one or two successive longitudinal divisions of the protoplast (Figs. 8-10). At maturity each daughter-cell becomes provided with four flagella whilst

still enveloped by the mother-cell-wall, which by this time is much distended, though fitting fairly close round the daughter-cells.

As to the affinity, the new organism no doubt comes near *C. multifilis* (Fres.), Dill.¹

It differs, however, from its nearest congener, firstly in its thicker cell-wall, secondly in its less round shape, and thirdly in its shorter flagella. According to Dill² the flagella of *C. multifilis* are inserted in pairs, and they do not radiate in four directions, as described by Goroschankin.³ He also states that *C. multifilis* possesses a flat papilla. The writer has not been fortunate enough to examine any reliable specimen of *C. multifilis*; but so far as his knowledge goes, in *Carteria*, *Chlamydomonas*, and possibly in the allied genera, the flagella (either four or two, as the case may be) are usually attached to the anterior protoplasmatic beak, from which they radiate in four, or, in the case of only two, in two directions, but not as described and figured by Dill. Unfortunately Dill does not give any figure of *C. multifilis* seen from the anterior end. If his statement was based on the examination of the side views only of the organism there is sufficient room for doubt. As to the structure referred to by Dill as a papilla,⁴ the writer differs in opinion. Judging from the figure given by Dill, the so-called 'Hautwärtchen' is only a thickening of the cell-wall, but not a papilla in the proper sense.

Dangeard apparently examined a form very similar to our new species,⁵ but confused it with *C. multifilis*, and consequently he proposes to modify Goroschankin's description of *C. multifilis*. It appears that the specimen delineated by Dangeard in his Fig. 19, C, had the inner gelatinous part of the cell-wall well developed at the anterior end, which apparently made him suppose that the protoplast had contracted.

Amongst the described members of the genus *Carteria*, *C. Klebsii* (Dang.), France,⁶ appears to be the only species which possesses a relatively thick cell-wall. The cell of this species is, however, ellipsoid or cylindrical, and is furnished with a conical papilla at the anterior end. Also the flagella of this species are, according to Dangeard, markedly shorter than the length of the body.

As to the mode of locomotion, it has already been pointed out that the organism usually swims forwards, but occasionally backwards. When it moves backwards the speed is not so great as that of the forward move-

¹ Dill: in Pringsh. Bot. Jahrb., vol. xxviii, 1895, pp. 341, 353.

² l. c., p. 342, Taf. v, Fig. 51.

³ In Bull. Soc. Imp. Sc. Nat. Moscou, 1891, p. 121. The organism is described under *Chlamydomonas*.

⁴ l. c., p. 353.

⁵ Dangeard: in Le Botaniste, 6^e sér., 1899, p. 159, Fig. 19 A, c.

⁶ France: Zur Systematik einiger Chlamydomonaden, 1892. This species was first described as *Pithiscus Klebsii*, Dang., in Ann. Sc. Nat., 7^e sér., vii, 1888, p. 137, Pl. 12, Figs. 1-6.

ment. The flagella seem to be stretched almost straight (cf. Fig. 3) and are often plainly visible. On the other hand, during the forward movement the flagella are scarcely visible.

Since the first note¹ on the backward movement of certain Chlamydomonads was written, the writer has had opportunities of examining living specimens of several species of *Chlamydomonas* and *Carteria*. It has been found that in most cases these organisms occasionally swim backwards. It appears that this kind of movement is fairly general amongst the Chlamydomonads, although it is possible that certain species do not swim backwards at all. Even amongst those which occasionally move with the posterior end forwards, some show this feature more frequently, or swim for a greater distance than others.

DIAGNOSIS.

Carteria Fritschii, Tak., sp. nov. (Figs. 1-10). Cellulae vegetativae (= zoogonidia) parvae, ovoideae vel subglobosae, interdum obovoideo-ellipsoideae, raro a latere paulo compressae; membrana cellularum exteriori firma ad $\frac{3}{4}$ μ crassa, in polo anteriori paullulum producta, sed sine papilla, membrana interiori (= parte membranae gelatinosa) saepissime valde et inaequaliter evoluta. Flagellis corpore cellulae paulo longioribus, e rostro plasmatice minutissimo in quatuor directiones radiatis. Chromatophora singula, viridis, urceolata, granulata; pyrenoide singulo, sphaeroideo vel subanguloso, conspicuo, in parte posteriori cellulae posito; stigmatibus ovali in parte anteriori cellulae; vacuolis contractilibus duobus, anterioribus; nucleo in parte subanteriori cellulae.

Propagatio: cellulae matriciales in unam vel duas directiones longitudinales in duas vel quatuor dividit.

Long. cell. 15-20 μ ; lat. 11-19 μ . Long. flagell. 17-22 μ .

Grege *C. multifili* (Fres.), Dill. collocanda, a qua cellula minus rotundata, membrana exteriori crassiori, membrana interiori saepissime bene evoluta, flagellis brevioribus satis distinguitur.

Hab. In sphagnis, Keston, Kent (F. E. Fritsch, 1915).

The writer wishes to express his thanks to Sir David Prain, C.M.G., C.I.E., for allowing him to carry out the present investigation in the Jodrell Laboratory, Royal Botanic Gardens, Kew. He also takes this opportunity to thank Professor G. S. West, of Birmingham, for valuable help.

¹ Takeda: Ann. Bot., vol. xxx, No. cxvii, January, 1916, p. 157.

On a Species of *Chlamydomonas* (*C. sphagnicola*,
F. E. Fritsch and Takeda—*Isococcus sphagnicolus*,
F. E. Fritsch).

BY

F. E. FRITSCH

AND

H. TAKEDA.

With fourteen Figures in the Text.

IN the first of the 'Notes on British Flagellates',¹ published by one of us, a new genus of green Flagellates was described under the name of *Isococcus*. A further examination of more abundant material, collected in May of last year and since kept in the laboratory, has shown that there were certain errors in the original description of this organism. Moreover, the facts that have come to light and are detailed below indicate that the organism in question does not constitute a distinct genus, but is a somewhat peculiar species of *Chlamydomonas*, which must pass under the name of *C. sphagnicola*. Whilst the material upon which the new observations were made was collected from the same marsh at Keston (Kent) from which the organism was first obtained, it may be mentioned that this species of *Chlamydomonas* has since been discovered also in one of the ponds in Richmond Park (Surrey).

The motile individual of *C. sphagnicola* is rather variable in shape. As a rule it is broadly ellipsoid (Figs. 1-3); not rarely, however, it is ellipsoid or oblong-ellipsoid, with more or less pointed or rounded poles (cf. Figs. 5, 8, 9, 10, 12), whilst in a few cases the individuals are subglobose or even globose (Fig. 6). When viewed from the anterior or posterior end the organism is circular (cf. Fritsch, loc. cit., Fig. 1, D and G), but if front and side views of the same individual are compared (Figs. 7-9, 13) they are seen to show slight differences in shape. The length (excluding the papillae) varies between 15μ and 27μ , the breadth between 9μ and 22μ (up to 25μ broad according to the old description, loc. cit., p. 341). The cell-wall is remarkably thick, and consists of an outer firmer portion and a usually well-developed inner gelatinous portion (sometimes as much as 4μ thick) which, in the previous description (loc. cit., p. 341), was regarded

¹ New Phytologist, vol. xiii, 1914, pp. 341-6.

[Annals of Botany, Vol. XXX. No. CXIX. July, 1916.]

as a space. The outer firmer part of the wall is very conspicuous, and reaches a thickness of about $1\ \mu$. By using suitable stains such as acid fuchsin, safranin, erythrosine, or aniline blue, the inner gelatinous part is readily brought to view. In some cases it is more strongly developed at the posterior end of the cell (Figs. 4, 5, 8–10), a state found also in other species of *Chlamydomonas* and *Carteria*. Specimens have also occasionally been found in which the gelatinous layer is thickest at the anterior end (Fig. 7). In some cases this gelatinous part of the wall contains granules of an unknown substance¹ (cf. Fritsch, loc. cit., p. 344, and Fig. 1, F).

The protoplast in living individuals is in direct contact with the inner gelatinous part of the cell-wall. In plasmolysed specimens a narrow clear zone is sometimes distinctly visible between the gelatinous layer and the protoplast.

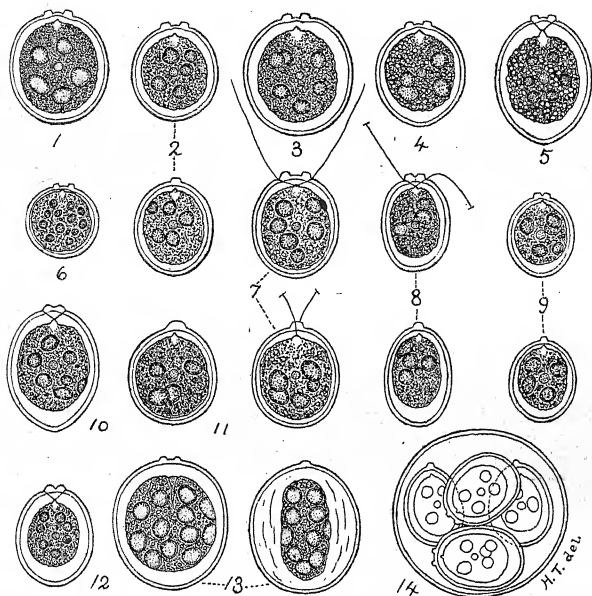
At the anterior end of the cell there are ordinarily two papillae (Figs. 1–7) which were wrongly interpreted as ciliary apertures in the previous account (loc. cit., p. 341). These papillae are, however, *solid* projections of the membrane, and the flagella do not pass through them, but emerge from the base on the abaxial side of each (cf. Figs. 7, 8, 10, 12). Whilst a considerable percentage of individuals exhibit two perfectly distinct papillae, all stages have been found connecting such forms with individuals in which there is but a single papilla (Fig. 11); the individuals shown in Figs. 8, 9, 10, and 12 illustrate intermediate conditions in which there is a single, more or less two-lobed, papilla. In shape the papillae are generally conical, with a rounded-truncate apex, although in some cases the conical character is less pronounced than in others. The papillae reach a height of $1\text{--}1.5\ \mu$. As far as we are aware this is the only known species of *Chlamydomonas* in which the papilla exhibits this character. We have not observed any further instances of the projection of the papillae on the inner side of the membrane as recorded in the earlier account (p. 342).

The surface of the protoplast is always more or less irregular, appearing unevenly crenate in optical section (Figs. 1, 4, 5) and often showing short faint lines when viewed from the surface. These features lead to a false impression of striation of the protoplast, but the phenomenon is most probably due to granulation of the surface. Occasionally the lines show a certain regularity, analogous to that depicted in the figures in the previous account, but the individuals examined by us never exhibited true spiral striation, and it is likely that the earlier description was in error in this respect.

At the anterior end the protoplast shows a well-marked colourless beak (loc. cit., p. 341), to the apex of which the two flagella are attached

¹ A similar feature has been observed in *Carteria Fritschii*, Tok., in which the inner gelatinous part of the cell-wall is often very well developed. For a description of this species see p. 369.

(Figs. 5, 7, 8, 10, 12). Below this beak lies a V-shaped colourless area, indicating the apex of the colourless protoplasmic mass enclosed within the central hollow of the massive chloroplast. The flagella are relatively coarse, and readily seen both in the living organism and in individuals fixed in



Chlamydomonas sphagnicola. $\times 1,000$. Figs. 1-12. Vegetative cells (= zoogonidia). Fig. 13. Very early stage in the formation of daughter-cells. Fig. 14. Four mature daughter-cells within mother-cell-wall. In Figs. 7-9 and 13 both front and side views are shown. Also in Fig. 14 two of the daughter-cells are viewed from front, while the other two are seen from side. Figs. 1-11 and 13-14 were drawn from fresh material collected at Keston in May, 1915, while Fig. 12 represents one of the individuals in the original preparations of *Isococcus sphagnicolus* collected in the same locality in May, 1914.

osmic acid. They are always longer than the cell, and sometimes reach a length of about one and a quarter times that of the body.

Two contractile vacuoles, which pulsate alternately, have been observed at the anterior end of the cell, just below the protoplasmic beak. There is a conspicuous stigma, lenticular in shape and somewhat anterior in position

D d

(Fig. 7; cf. also loc. cit., Fig. 1, C). The chloroplast is a massive structure of a dark green colour which, as already mentioned, occupies the greater part of the cell. Four or more pyrenoids, more or less globular in shape and quite irregularly arranged, lie in a parietal position. The pyrenocrystal and amylaceous envelope can easily be differentiated by means of the usual stains. The small nucleus is situated in the centre of the cell.

To the earlier description of the formation of daughter-cells (p. 344) we are able to add but little. Prior to division, the pyrenoids seem to increase in number (cf. Fig. 13). At the same time the gelatinous part of the cell-wall appears to acquire a very thin consistency. The first division is longitudinal, and takes place in a plane which is at right angles to that containing the two papillae, the constriction of the protoplast starting at the anterior end (Fig. 13). The second division, when it takes place, appears likewise to be longitudinal, but runs at right angles to the first division-plane. By the time that the daughter-cells have acquired their mature characteristics, their arrangement becomes irregular (Fig. 14), which appears to be due to active movements on the part of the new individuals (cf. also Fritsch, loc. cit., p. 344). We did not observe any further instance of division into eight (cf. Fritsch, loc. cit., p. 344). Prior to liberation of the new individuals the mother-cell-wall swells up very considerably and acquires a very thin consistency.

As regards the affinity of *C. sphagnicola*, it may be compared with *C. longistigma*, Dill, *C. gloeocystiformis*, Dill, *C. angulosa*, Dill, and *C. gigantea*, Dill.¹ The first of these species (Dill, loc. cit., pp. 328, 354, Tab. V, Figs. 1-8) has a broad and flat papilla, somewhat recalling that found in the specimens of *C. sphagnicola*, in which but a single papilla is present (cf. above). It differs from our species in having a thin membrane, without any gelatinous layer, a smooth surface to the protoplast, and a long rod-like stigma.

C. gloeocystiformis, Dill (loc. cit., pp. 340, 354, Tab. V, Figs. 37, 38), has a thick gelatinous membrane, in which an outer firmer portion and an inner gelatinous part could perhaps be distinguished, although Dill does not discriminate between the two, either in his description or in his figures. The species differs from *C. sphagnicola* in having but a single papilla and a solitary pyrenoid which is not parietal.

C. angulosa, Dill (loc. cit., pp. 337, 354, Tab. V, Figs. 21-25), has a distinct papilla which is broadly conical in shape. The cell-wall is usually thin, and the gelatinous layer is only seldom developed to a slight extent at the posterior end. Moreover, this species possesses one, or rarely two, pyrenoids, which are axial, and a smooth protoplast.

C. gigantea, Dill (loc. cit., pp. 338, 353, Tab. V, Figs. 25A-30), resembles *C. sphagnicola* in having a considerable number of pyrenoids, which here,

¹ Cf. Dill: Die Gatt. *Chlamydomonas*, &c. Pringsh. Jahrb., vol. xxviii, 1895.

however, are not parietal but lie near the inner face of the chloroplast. Moreover, this species has a very thin cell-wall, without any gelatinous layer, and a poorly developed papilla. The stigma is situated near the centre of the cell, and is often very long.

The most striking feature presented by *C. sphagnicola* is certainly the customary doubling of the papilla at the anterior end of the cell. A somewhat similar condition has been recorded and figured by Wollenweber¹ in the case of certain species of *Haematococcus* (= *Sphaerella*), in which the flagella occupy the same position with reference to the two papillae as in our species. It can hardly be doubted that the paired papillae are the result of fission of the single structure normally present in other species of *Chlamydomonas*, &c., and occasionally even developed as such in *C. sphagnicola*.

In conclusion, we append a Latin diagnosis:

Chlamydomonas sphagnicola (Fritsch), Fritsch and Takeda, comb. nov. (Figs. 1-14).

Syn.: *Isococcus sphagnicolus*, F. E. Fritsch, in New Phyt., xiii, 1914, p. 351, Fig. 1.

Cellulae vegetativae (= zoogonidia) pro genere magnae, late ellipsoideae, non raro ellipsoideae vel oblongo-ellipsoideae, polis aut rotundatis aut subacutis, interdum subglobosae vel globosae. Membrana cellularum crassissima, pars exterior firma ad 1μ crassa, pars interior gelatinosa plerumque bene evoluta, ad 4μ crassa, polo anteriore papillis duabus vel tantum papilla singula plus minus biloba vel rarissime integra praedita. Flagella ca. ad $1\frac{1}{2}$ plo longiora quam corpore cellulae, rostro protoplasmatica affixa. Chromatophora singula, urceolata, viridis, densissima, granulata; pyrenoidibus parietalibus, 4 vel pluribus, plus minus globosis, conspicuis; stigmatibus conspicuis, lenticulari, fere anteriori; vacuolis contractilibus duabus; nucleo centrali. Propagatio subdivisione cellulae matricialis longitudinaliter in 2, vel 4, rarissime 8 partes fit.

Hab.: In Sphagnis, Keston, Kent (F. E. Fritsch, May, 1914-May, 1915); in stagno, Richmond Park, Surrey (H. Takeda, December, 1915, January, 1916).

The greater part of the present reinvestigation was carried out in the Jodrell Laboratory, Royal Botanic Gardens, Kew. We take this opportunity of expressing our thanks to Sir David Prain, C.M.G., for the facilities afforded to us.

¹ Ber. Deutsch. Bot. Ges., xxvi, 1908, p. 245, Fig. 3a and b, Fig. 7, and Tab. XIII, Figs. 1, 5-8.

Studies on Nuclear Division in Desmids.

I. *Hyalotheca dissiliens* (Sm.) Bréb.

BY

ELIZABETH ACTON, M.Sc.

With Plate VIII and four Figures in the Text.

INTRODUCTION.

THE only account which has so far appeared of the division of the nucleus in Desmids is that by Lutman,¹ in which he describes the cell- and nuclear division in *Closterium*.

It was therefore suggested by Professor G. S. West that this subject was a suitable one for investigation, and the present contribution is intended to be the first of a series of papers dealing with the nuclei and nuclear division of Desmids.

The fact that very little is known of the cytology of Desmids is probably largely due to the difficulty of obtaining suitable material to work upon. It is by no means an easy matter to obtain a continual supply of any of the larger Desmids. They usually occur in places which are not easily accessible, and when brought indoors they cannot be kept for any length of time in a healthy condition. The ordinary culture-methods which serve for the common filamentous Algae are a complete failure as far as Desmids are concerned, and the conditions under which they divide or form zygospores seem to be so highly specialized that it has not been possible to determine them up to the present time. So that, while it is possible accidentally to discover Desmids in quantity in a dividing condition, the possibilities of finding them at any particular time or inducing them to divide by artificial means are rather remote.²

Hyalotheca dissiliens is the only Desmid I have been able to obtain, so far, in sufficient quantity to give complete results, and it is for this reason alone that it has been chosen for the initial paper. Unfortunately, the nucleus in *Hyalotheca* is too small to give entirely satisfactory results.

No attempt will be made in this paper to compare the results obtained with those of Lutman or to discuss their bearing with regard to other Conjugatae. I hope to complete shortly the investigation of several of the

¹ B. F. Lutman : Cell and Nuclear Division in *Closterium*. Bot. Gaz., 51, 1911.

² Some of the larger species of *Closterium* are an exception to this statement, as they will grow and multiply rapidly in large tanks in greenhouses, &c., and will also survive culture experiments.

larger Desmids, and all discussion will be deferred until these results can be summarized.

TECHNIQUE.

Material was at first fixed in weak Flemming's mixture diluted to half strength, as Lutman found this the most satisfactory fixing reagent for *Closterium*. It did not give entirely satisfactory results in the case of *Hyalotheca*. There was not much shrinkage of cell contents, but staining results were not good after using this reagent, possibly owing to the thick gelatinous sheath which is present in *Hyalotheca*. Stronger fixing reagents were tried, and of these Bouin's picroformol solution was found to give the best results. This solution therefore has been tried for all the later work. Heidenhain's iron-alum-haematoxylin was used for staining. In order to prevent shrinkage it was found necessary to exercise special care in transferring the stained material from xylol to Canada balsam. The material was transferred from xylol to a solution of Canada balsam diluted with xylol to about one-fifth of its original strength. This was then allowed to evaporate slowly until it reached its original strength.

Collections of *Hyalotheca* were made during the winter and spring and dividing filaments occurred in every collection. The greatest number of dividing filaments was present in material fixed towards midnight, but plenty of dividing filaments were to be found in material fixed as early as 4 p.m.

RESTING CELL AND NUCLEUS.

In the resting state the cell contains two chromatophores with the nucleus lying between them (Pl. VIII, Fig. 2). Seen from the end each chromatophore is star-shaped, the centre of the star being formed by a large pyrenoid from which the plates of the chromatophore appear to radiate (Fig. 1). The starch-sheath of the pyrenoid is very large, and is made up of separate plates. The number of pyrenoids is not constant. Filaments in which every chromatophore contained two pyrenoids were of frequent occurrence, and filaments with chromatophores containing three and four pyrenoids were sometimes found. Their arrangement in a transverse row in the cell suggests that they are formed by longitudinal division of the existing pyrenoid, but no division of pyrenoid was ever observed except the transverse division of the pyrenoid during division of the cell; so that it is impossible to state whether these extra pyrenoids arise by division of existing pyrenoids or *de novo*.

The nucleus is spherical in shape; though in stained preparations it often appears to be somewhat flattened by pressure from the pyrenoids. The nucleolus is comparatively large and stains deeply with chromatin stains. The reticulum is inconspicuous, stains faintly, and appears to have few, if any, chromatin granules.

DIVISION OF NUCLEUS AND CELL.

The first sign of division in the nucleus is the appearance of granules on the nuclear network, which consequently shows up more clearly on staining (Fig. 3). At the same time the nucleolus stains less deeply and finally disintegrates. The small size of the nucleus makes it exceedingly difficult to follow exactly what happens in the succeeding stages. The granules appear to increase in size and decrease in number, so that probably the chromosomes are being formed on the spireme. The gradual disappearance and disintegration of the nucleolus makes it improbable that the chromosomes come bodily out of the nucleolus.

The next stage that could be definitely seen was the collection of the chromosomes on the equatorial plate (Pl. VIII, Fig. 6). The chromosomes are short broad rods, almost granules, and about twelve in number. A definite spindle could not be seen, but fibres could clearly be seen attached to the chromosomes and pulling the daughter chromosomes to the opposite poles of the nucleus (Figs. 7 and 8).

In the reconstruction of the daughter nuclei the chromosomes lose their identity, granules appear in the nucleus, and these gradually fuse to form the large nucleolus (Figs. 9-12).

The daughter nuclei begin to separate immediately they are formed, and to move in opposite directions towards the surface of the cell, where they take up a position opposite the pyrenoid.

Formation of the new cell-wall was not observed in detail. It is always completed before the division of the chromatophore begins, so that for a time the cell has only one chromatophore.

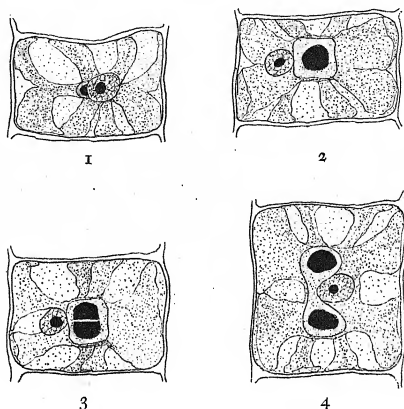
As the chromatophore divides the nucleus slips in between the two halves until it finally reaches the pyrenoid and, during the division of the pyrenoid by constriction, remains firmly pressed up against it. The division of the chromatophore and pyrenoid is probably largely influenced by the presence of the nucleus. No trace of division appeared in the chromatophore until the nucleus had moved round to the surface of the cell and taken up its position opposite the pyrenoid. Constriction does not take place equally all round the chromatophore, division always being further advanced in the part in which the nucleus lies (see Text-figs. 1-4, p. 382).

The division of the pyrenoid and chromatophore has already been figured and described by Schmitz,¹ but the close connexion between the division and the presence of the nucleus was not noted.

The starch-sheath of the pyrenoid seems to be fairly intact at the completion of the division of the nucleus, though signs of disintegration have already appeared. It is not until the beginning of division in the chromatophore that it decreases rapidly in size, and at the end of this process no trace of the starch-sheath is visible.

¹ Schmitz, F.: Die Chromatophoren der Algen. Verhandl. d. naturf. Ver. Bonn, Bd. xl, 1880

The fact that the starch-sheath does not noticeably diminish in size during division of the nucleus, but only during division of the chromatophore, suggests that the presence of a large quantity of starch in the cell is not in itself sufficient to induce division. The presence of two or more



Illustrating successive stages in the division of the chromatophore and pyrenoid.

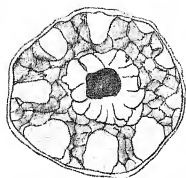
pyrenoids in the cell is probably due to the fact that, although the conditions necessary for active starch-formation are present, the extra conditions necessary to induce nuclear division are absent, and additional pyrenoids are therefore formed to carry off the surplus starch.

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BIRMINGHAM.

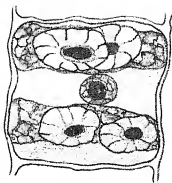
DESCRIPTION OF PLATE VIII.

Illustrating Miss Acton's paper on *Nuclear Division in Desmids.*

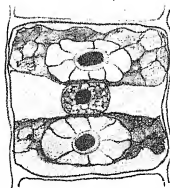
- Fig. 1. End view of cell, showing chromatophore and pyrenoid. $\times 1,450$.
 Fig. 2. Resting cell and nucleus. $\times 1,450$.
 Figs. 3-5. Gradual disappearance of nucleolus and probable formation of spireme. $\times 1,450$.
 Fig. 6. Appearance of chromosomes. $\times 2,000$.
 Fig. 7. Metaphase. $\times 2,000$.
 Fig. 8. Anaphase. $\times 2,000$.
 Figs. 9 and 10. Telophase. $\times 1,450$.
 Fig. 11. Daughter nuclei beginning to migrate to the surface. $\times 1,450$.
 Fig. 12. Nucleolus re-formed in the daughter nuclei. $\times 1,450$.



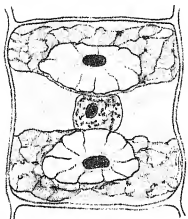
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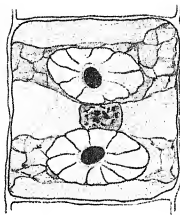
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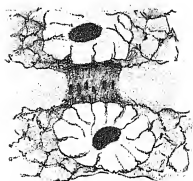
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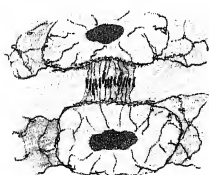
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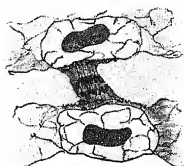
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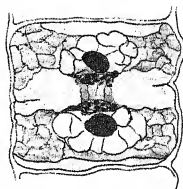
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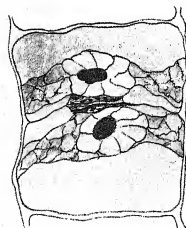
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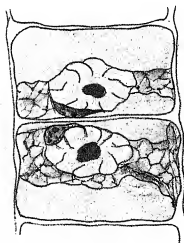
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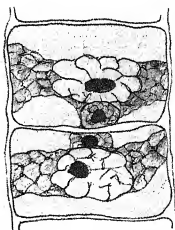
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Huth, London.

ACTON - HYALOTHECA DISSILIENS.



On the Supposed Origin of Life in Solutions of Colloidal Silica.

BY

SYDNEY G. PAINE.

With Plate IX.

THE origin of life is at present in entire obscurity, and it would seem that our knowledge of chemistry and physics must advance considerably before any real light can be thrown upon it.

It is a question which lends itself more to speculation than to laboratory practice, but it is hoped that one day we may be in a position to investigate experimentally the phenomena concerned in the change from the non-living to the living state.

Except for certain investigations in the first half of the nineteenth century, the only experimental work which definitely had as its object the realization of this change is that of the late Dr. Charleton Bastian. Since the appearance of his first publication on the subject in 1870 until the time of his death, Dr. Bastian held firmly to the view that living organisms may arise *de novo* from non-living materials. During the past twenty years he has supported his view by numerous experiments in which he describes the development of organisms under conditions calculated to exclude all possibility of infection by a living germ.

The results of this work are to be found in three monographs by this author (1), and in the pages of *Nature* (2).

The method of experiment is described fully at page 30 of 'The Origin of Life', and consists of enclosing in special tubes very dilute solutions of colloidal silica mixed either with phosphoric acid or with some form of colloidal iron; after sealing, the liquids are carefully sterilized by intermittent sterilization at 100° C. or by short exposures to temperatures of 120° C. to 135° C. Thus prepared, the tubes are exposed to light at an east window for periods varying from six months to two years. During this time a small deposit collects at the bottom of each tube, and the examination of this is made by removal with a fine pipette on to a microscope slide, or the liquid is sometimes centrifuged and the deposit examined microscopically.

According to Dr. Bastian many forms of Bacteria, Torulae and fungal hyphae are to be found in the amorphous matrix which this deposit always presents.

Dr. Bastian claims that successful infections can be made of these upon various nutrient media. In this he is supported by A. and A. Mary (3), and also by A. H. Drew (4), who, according to Dr. Bastian, has succeeded by the use of tyrosine in cultivating bacilli more or less freely. On the contrary, J. Wright in New York (4) repeating these experiments has obtained similar structures, but has been unable to obtain growth of these on artificial media.

In 1913 Dr. Bastian showed some of his supposed organisms to many biologists, amongst others to Professor Farmer and Professor Blackman at the Imperial College of Science and Technology, who, in fairness to him and in view of the fact that his later methods made sterility more probable than was the case in some of his earlier experiments, desired that a careful repetition of the experiments should be made. At their instigation the author commenced, as stated in a note in Nature (5), an investigation in order to discover if possible the origin and nature of the organisms described.

Experiments have been carried on now for more than two years, and altogether eighty-five tubes have been under examination. These tubes were prepared in accordance with the directions given by Dr. Bastian in his book 'The Origin of Life', and contained a mixture of dilute solutions of colloidal silica, ammonium phosphate and phosphoric acid, or a mixture of colloidal silica with liquor ferri pernitratis; the former he speaks of as the *colourless solution*, the latter as the *yellow solution*. In addition, during last year a few experiments were made with mixtures of colloidal iron, potassium ferrocyanide and sodium silicate, which Dr. Bastian found to be specially fruitful. The conditions of experiment have followed as closely as possible those of Dr. Bastian's experiments; the tubes were sealed and sterilized according to his instructions and exposed to light at an east window.

When examined six to eight months after sterilization, the majority of the tubes showed deposits consisting mainly of an amorphous mass of silica together with minute shining plates, also presumably of silica.

There also occur very frequently small highly refracting spheres either scattered singly in the amorphous matrix or collected together in masses. They are in size and appearance very similar to the bodies described by Dr. Bastian as *fungus germs*.

A very large deposit of these bodies was found in a 10 per cent. solution of sodium silicate which remained tightly stoppered in a 100 c.c. flask from February to October of last year. The deposit had formed a loose layer 3 to 4 mm. thick at the bottom of the flask. The microscopic appearance of these bodies is shown in the accompanying photograph

(see Pl. IX, Fig. 1), and seems to be identical with that figured several times in Dr. Bastian's book on 'The Origin of Life,' notably with that in Pl. I, Figs. 2 and 6; Pl. II, Fig. 8; Pl. IV, Fig. 23; and Pl. X, Fig. 57.

After microscopical examination the remainder was collected on a tared filter, and when dried at 100°C. weighed 14.4 mgm. Analysis showed it to consist of 38.9 per cent. SiO_2 , a very small trace of Fe_2O_3 , and a trace of Na_2O . No organic matter was present, as shown by absence of charring on heating with H_2SO_4 . The remaining 60 per cent. was presumably water. When heated the transparency of the material was lost, and the particles broke up to a fine white powder. The author concludes that they were little solid spheres of hydrated silica deposited from colloidal solution.

In some of the tubes larger rounded bodies have occurred; a mass of these surrounded by an amorphous deposit is shown in Pl. IX, Fig. 2. When crushed under the cover-slip these bodies separated from the matrix and were found to be flat discs or sacs of round or oval contour, varying in diameter from 10μ to 60μ . Being perfectly transparent objects they proved very difficult to photograph, but eventually a fair representation was obtained by varying the intensity of light in different parts of the field. The results are given in Figs. 3 and 4.

Many of the bodies appear to have budded and have much the appearance of yeast cells. This is probably caused through cohesion of the smaller with the larger particles. These bodies are probably little discs or solid spheres rather than hollow sacs or cells, and any yeast-like budding would thus seem to be out of the question. Evidence for this view is afforded by one or two specimens shown in Figs. 3 and 4, which have been broken by pressure on the cover-glass in mounting, and which exhibit irregular fracture strongly indicative of such solid nature. It is not suggested that these large bodies were exactly the same as those described by Dr. Bastian as *Torulae*, but they seem to differ only in point of size from those which he figures in Pl. VIII, Figs. 45 and 47 B of his book.

The author is indebted to the late Dr. Bastian for supplies of materials used by him, and further for the opportunity he gave the author of seeing some of his own preparations and of opening and examining three of his own tubes from a series of experiments which had given him positive results.

From one of these tubes sealed July 8, 1914, opened in December, 1914, a large number of round bodies were obtained from a gelatinous deposit which had collected on the sides of the tube just above the level of the liquid. As first examined they had the appearance shown in Fig. 5.

By simple manipulation with a needle a few of these were separated from the amorphous residue, washed well with water, and allowed to dry on a thin glass slide, upon which they were heated to dull redness.

As the result of this treatment no charring was observed, and the

bodies seemed to remain entirely unaltered. They were then mounted in a resinous medium (euparal) and examined closely with a high power objective. They appeared as thick-walled hollow spheres apparently of silica, the walls bearing pits very similar to those in the walls of the so-called *stone cells* of the pear. The pitting is well shown in the original photograph, but this detail will probably disappear in the process of reproduction. In Fig. 6 three of these objects are seen in juxtaposition, and beneath these several fragments of others which have been broken by pressure on the cover-glass during mounting. The objects were very brittle and, as stated above, were hollow; to this fact is due the apparent blackness of one of the spheres from which the air was not expelled by the mounting fluid.

Comparison of these preparations with Dr. Bastian's Figs. 5 and 35 in 'The Origin of Life' points to the conclusion that the bodies under investigation are identical with those figured by him. The granular contents of which he speaks in the description of Fig. 5 appear to the author to be only pits in the walls such as he has observed in his own preparations.

The method of formation of these bodies is so far obscure.

In contrast, the method of formation of the other silico-morphs mentioned above, namely the small solid spheres and the flat discs, is not difficult of explanation. These bodies have probably been built up by slow deposition from a colloidal solution upon minute specks of solids, or upon nuclei composed of the first aggregates which have separated from such solution. That silica is deposited slowly from colloidal solutions upon solids immersed in them is well shown in Pl. IX, Fig. 7. This specimen was found in a bottle of colloidal silica which had been left undisturbed for twenty-one months. The nucleus for deposition in this case seems to have been a cotton fibre which in course of time has become irregularly coated with a hard glass-like mass of silica. Fine markings visible in the original photograph, which appear as striae in the deposit, will probably be lost in reproduction.

Besides the forms already mentioned, there occurred in most of the tubes very fine threads with something of the appearance of fungal hyphae. These have been well figured in a recent publication by Professor Moore and W. G. Evans (Proc. Roy. Soc., 1915, B., 89, p. 17), who have shown that they result by slow decomposition from metastable solutions of inorganic colloids. That they are not threads of fungal mycelium is very apparent when, while under observation with a low power objective, an attempt is made to draw them across the slide with a fine pointed needle. Thus in several experiments of this kind it was observed that the thread is not carried along or bent by the needle, as would be the case with a fungal hypha, but is ruptured by the needle at the point of contact, the substance of the thread being drawn out in a streak of gelatinous material.

Moreover, when attempts were made to stain these threads it was

found that they stained uniformly with various dyes, but showed none of the contents which are so characteristic of living fungal hyphae. It is clear that the threads in question are merely deposits of colloidal material simulating only in a slight degree the hyphae of a fungus.

The author has never obtained in sterilized tubes any growths of a typical fungal or *Torula*-like nature, and no growth has ever developed in nutrient media which have been inoculated from the tubes at the time of opening. In cases, however, where the tubes were not sterilized at all, or where they were allowed to stand for a week before being sterilized, there have usually been found rudimentary mycelia of a very crumpled appearance. These were obviously dead, and surrounded by a deposited layer of silica. In most of these cases it is fairly easy to trace the spore from which the mycelium has developed, although this is not always possible. Fig. 8 represents one of these growths from a tube which was prepared, sealed, and left eight days before being sterilized by intermittent sterilization at 100°C . Here the spore is quite obvious, and there can be no doubt that similar growths must inevitably be introduced with the solutions if these should have been made even a few hours before being filled into the tubes.

The growth shown in Pl. IX, Fig. 9, was observed in a tube containing colloidal silica and ammonium phosphate which had been opened and left standing for five months. The microscopic appearance is strikingly similar to that represented in many of Dr. Bastian's figures. At the time of observation this fungus was dead and the cells, when treated with Delafield's haematoxylin, exhibited contents or some remains of organic material such as are typical of the disorganized contents of a fungal mycelium.

A very similar growth was also obtained in a solution of colloidal silica during the process of dialysing in a parchment dialyser which had not been sterilized.

That growths obtained under these conditions so closely resemble those found by Dr. Bastian would seem to suggest that the conditions under which circumstances forced him to labour were not always conducive to perfect sterility of his solutions.

All these observations support the view which has frequently been urged by others that the *organisms* described by Dr. Bastian were either purely inorganic silico-morphs, or else were produced by the deposit of silica on the surface of dead fungal hyphae or yeast cells which had developed in the solutions before these were filled into the tubes and sterilized.

This work was completed and ready for publication at the time of Dr. Bastian's death, and it is a matter of regret that he is no longer with us to take up the challenge. His work has aroused considerable interest in certain quarters, and it therefore seems advisable to place these observations on record.

SUMMARY.

1. The experiments of Dr. Bastian have been repeated.
2. In all, eighty-five tubes of colloidal silica have been examined.
3. Forms, which in a slight degree resembled organisms, have been found amongst the amorphous deposit which collected in the tubes, and these have been shown to be composed of silica.
4. These bodies are thought to be identical with some of the so-called *fungus germs* described by the late Dr. Bastian.
5. It is concluded that the forms resembling organisms, described by Dr. Bastian as evidence of spontaneous generation of life, were in part purely inorganic simulacra formed by slow deposition of silica from colloidal solution, and in part depositions of silica upon dead fungal hyphae which had developed in the solutions before these were filled into the tubes and sterilized.

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2. Nature, Jan. 22, and Dec. 24, 1914; July 15, 1915.
3. Le Médecin (Brussels), Oct. 31, 1913, and Jan. 15, 1914.
4. Nature, vol. xciv, 1914, p. 466.
5. Nature, Feb. 12, 1914.

EXPLANATION OF FIGURES IN PLATE IX.

Illustrating Mr. Paine's paper on *Colloidal Silica*.

- Fig. 1. Deposit of small spheres of silica from a solution of sodium silicate.
- Fig. 2. Deposit from a tube of colloidal silica, ammonium phosphate and phosphoric acid.
- Fig. 3. Discs of silica separated from Fig. 2 by pressure between the cover-slip and the slide upon which the deposit was mounted in water.
- Fig. 4. The same more highly magnified.
- Fig. 5. Deposit of gelatinous silica containing hollow spheres of silica removed from the sides of one of Dr. Bastian's tubes.
- Fig. 6. Three of the same objects separated from the amorphous matrix, heated on a slide and mounted in euparal. (The dark object contains a bubble of air.) Fragments of others appear in the lower half.
- Fig. 7. Cotton fibres upon which silica has been laid down by deposition from a solution of colloidal silica.
- Fig. 8. Dead fungal mycelium from a tube which had remained for a week before sterilization.
- Fig. 9. Fungi and spheres of silica from a tube of colloidal silica, ammonium phosphate and phosphoric acid, which was not sterilized.

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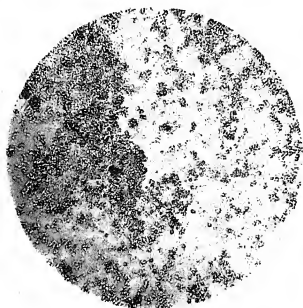
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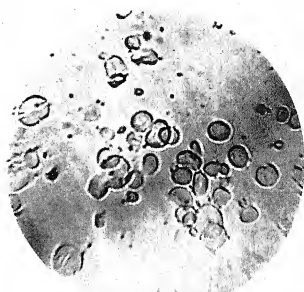
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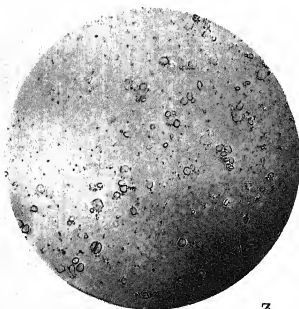
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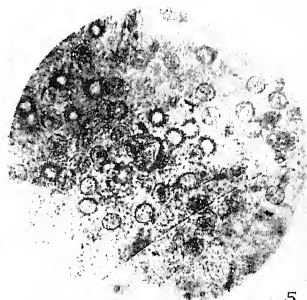
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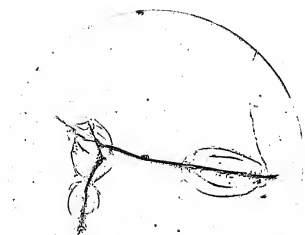


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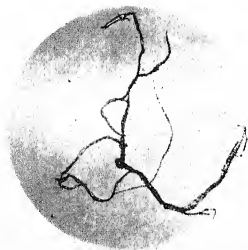
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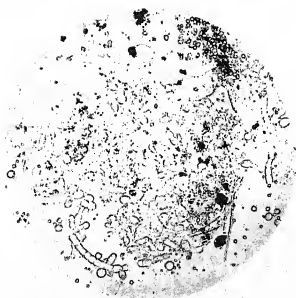
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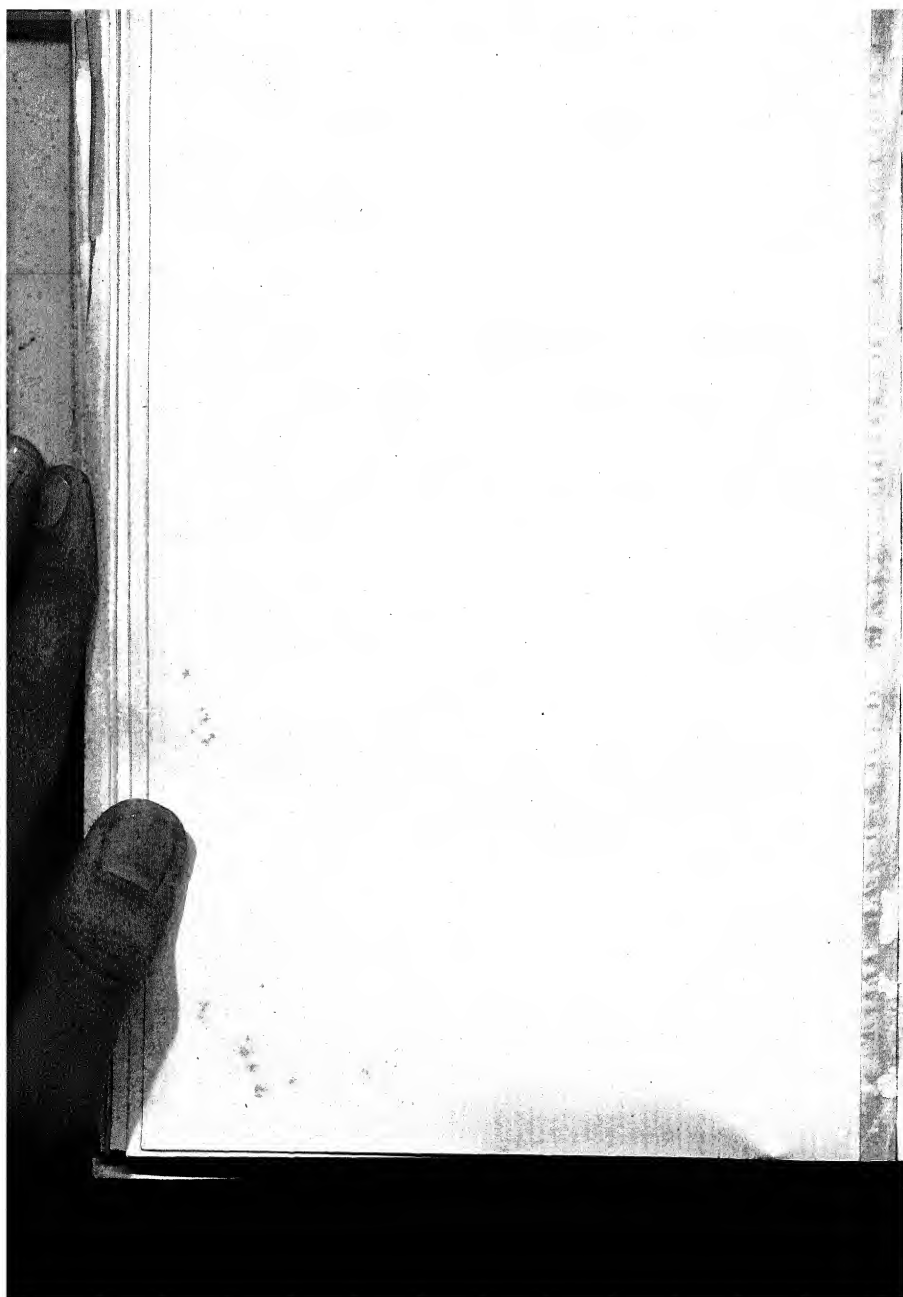
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Studies in the Physiology of Parasitism.¹

II. Infection by *Botrytis cinerea*.

BY

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AND

E. J. WELSFORD, F.L.S.,

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With Plate X and two Figures in the Text.

THE germ tubes of parasitic fungi which are not wound parasites usually infect the aerial parts of plants by entering through the stomata or by boring through the outer walls of the epidermal cells. In the case of stomatal infection the epidermal defences of the host plant are completely turned and infection is a comparatively easy matter, since many fungi possess enzymes which are able to cause disorganization of the cellulose walls of parenchyma cells.

Where, however, the cuticle is perforated, our knowledge of the mechanism employed by the germ tube is very meagre. Busgen (8) has pointed out the importance of appressoria in many cases in bringing the fungus in close contact with the host. Most authors assume, as apparently does Busgen, that the germ tube softens and dissolves the cuticularized epidermal wall in the same way as a cellulose wall. For example, Marshall Ward (11), in his classical paper on a disease of the Lily due to *Botrytis*, speaks of the germ tube as dissolving its way through the cuticle. Miyoshi (5, p. 286) is of opinion that the perforation of many membranes of fungi is due to the secretion of enzymes, although he had been able to show that *Botrytis cinerea* could perforate a membrane such as gold leaf, upon which it could by no possibility act chemically. Voges (10) speaks of the slime formed by the germ tube of *Fusicladium* softening the cuticle. Such a view, however, has never been supported by physiological evidence

¹ The first of this series of studies appeared in the *Annals of Botany*, vol. xxix, 1915, p. 313.

(*Annals of Botany*, Vol. XXX. No. CXIX. July, 1916.)

nor by any careful microscopic study of the actual phenomena of penetration. It is certainly improbable, since no enzyme is known which is able to dissolve cuticle.

A study (Brown, 1) in this laboratory of a powerful enzymic extract of the germ tubes of *Botrytis cinerea* had shown that such an extract was unable to exert any swelling or dissolving action on the tissues of a leaf when placed on the uninjured cuticle, although when injected into the leaf it rapidly brought about disorganization of the tissues. It seemed then advisable to make a careful study of the early stages of infection by *Botrytis cinerea*, paying particular attention to the phenomena to be observed in connexion with the penetration of the cuticle.

METHODS.

Cut leaves of the broad bean (*Vicia Faba*) were used as material for infection. The cultures of *Botrytis cinerea* used were from the strain employed by Brown (1) in his work. It was found that the spores germinated in water very slowly, or not at all, and very often failed to infect a leaf. For this reason the work has been carried out with spores sown in turnip juice on a leaf; this ensures rapid germination and strong, well-nourished hyphae.

The cultures were grown for ten days at 26° C. on sterilized potato-mush-agar.¹ The culture, which by this time has produced a plentiful crop of spores, is then flooded with water, and the surface of the medium gently scraped with a scalpel to detach the spores and mycelium. The suspension of spores and hyphae is passed through fine muslin to remove the mycelium, and then centrifuged for a few minutes. The water is then poured off very carefully and sterilized turnip juice is added—10 c.c. of turnip juice to every 0.1 c.c. of wet spores. The turnip juice is prepared by subjecting peeled and chopped turnips to a temperature of 120° in the autoclave for 45 minutes, and subsequently extracting the juice by means of a press. The suspension of spores in turnip juice is used for infection.² Infections were also made with a much less concentrated suspension of spores.

Before infection the leaves are washed with a gentle stream of sterile distilled water to remove as far as possible extraneous spores and dust. They are then placed on damp filter-paper on a sterile Petri dish, and drops of the prepared solution containing spores placed on their upper surfaces.

Material was fixed at intervals mostly in Flemming's fluid (the strong solution diluted with an equal bulk of water), and some in absolute alcohol containing 25 per cent. by volume of glacial acetic acid. It is easy to

¹ See Brown (1), p. 68.

² This method was worked out by Brown (1) in this laboratory; by its means an infecting suspension of standard strength is obtained. It has been shown that the spore concentration may markedly affect the degree of germination.

gauge roughly the stage of infection as, soon after penetration of the leaf, the tissues in the neighbourhood of the point of entry become brown and then black. The blackening is, of course, a well-known enzymic effect.

In studying later stages of penetration the sections were stained with gentian violet and orange G, or iron-alum-haematoxylin and erythrosin, or Delafield's haematoxylin and eosin (Durand, 4). In the very earliest stages of penetration iron-alum-haematoxylin was used, followed by scharlach red (in equal parts of glycerine and water); such preparations which were mounted in glycerine showed the cuticle stained very sharply. Gentian violet in dilute solution was found useful in demonstrating the stratification of the swollen subcuticular wall; the layering was particularly clear in preparations mounted in 'euparal'.

To demonstrate the outer mucilaginous layers of the walls of the germ tubes and hyphae, fresh material was mounted in 'collargol', a preparation of colloidal silver. The mucilaginous layer then appears as a clear area round the ordinary cell-wall. The mucilaginous coating can also be demonstrated by staining fresh material in dilute watery gentian violet and mounting in water.

OBSERVATIONS.

Germination. The dry conidia swell when placed in turnip juice and in 2-3 hours show the first signs of a germ tube. In a highly nutrient medium like turnip juice growth is very rapid, and in 15 hours the germ tube may reach a length of nine spore-diameters. If the germ tubes are growing in drops on a glass plate numerous appressoria will usually be formed in 24 hours, and at the end of 36 hours numerous cross-connexions are to be seen between the hyphae. These connexions and the interweaving of the hyphae convert the originally separate growths into a more or less felted mass.

The germinated spores show numerous nuclei; in the germ tube the nuclei are seen to be arranged in pairs (Pl. X, Figs. 6, 7, 8), and usually show conjugate divisions (Welsford, 12).

Anchorage of the germ tube. It was noticed early in the investigation that young germinated spores, even before any haustoria were developed, usually showed no tendency to become detached from the leaf as a result of the manipulation entailed in fixing, washing in running water, &c. The cause of the strong adhesion of the young germ tubes to the leaf was found to be the mucilaginous nature of the outer layers of the wall of the germ tube. This appears to have been overlooked by previous workers owing to its transparency; but it is easily demonstrated by mounting the germinating spores in a fine suspension of dark coloured particles. This method was first employed by Errera (5) to demonstrate the gelatinous sheath round filamentous algae. Instead of the Indian ink used by Errera a preparation

of colloidal silver, as already stated, was employed. The gelatinous or mucilaginous sheath round the germ tube then shows itself as a clear halo against a brown background (Fig. 5). The sheath is also easily demonstrated by staining with weak gentian violet for thirty seconds and mounting in water.

The mucilaginous envelope cannot be demonstrated in the earliest stages of germination (Pl. X, Figs. 1, 2); in such stages the germ tubes do not become fixed to the substratum, such as a glass slip, on which they are growing. Usually it is only when the germ tube has reached a length equal to the spore-diameter that it is able to adhere to the substratum; at this stage a mucilaginous sheath can be demonstrated (Fig. 3). In later stages, eighteen hours after sowing in turnip juice, the mucilaginous sheath is still more obvious (Fig. 4). The mucilage forms a very thin layer round the tip of the germ tube, but at the basal end does not reach quite up to the spore (Figs. 3 and 4).

The wall of the young germ tube before the appearance of a mucilaginous sheath appears to be thicker than the inner *non-mucilaginous* layer of the wall of the older germ tube; this gives support to the view that the swelling is due to the gelatinization of the outer layers.

In fixed and stained preparations the mucilaginous material no longer appears as a continuous sheath, but is reduced to a number of fine granular threads (Figs. 6, 7), which connect the germ tubes and spores to the substratum, and also to one another if the germinating spores occur in close proximity. The threads, as such, are clearly artefacts, and are in all probability mainly the result of the action of dehydrating agents, such as alcohol, on the continuous mucilaginous material.

No mucilaginous sheaths are to be seen in fresh material round the actual spores, whereas in fixed and stained preparations threads are sometimes to be seen connecting not only the germ tubes but also the spores (Fig. 8) to the substratum. The result appears to be simply explained by the more or less general distribution of the mucilage throughout the drop; for such threads are also to be found connecting one germ tube to another and also connecting a germ tube to another spore. In support of this explanation it is to be noted that fluid in which spores are germinating is mucilaginous to the touch.

A thick mucilaginous layer can also be demonstrated round the group of hyphae forming the appressoria which develop so readily on a glass surface (Fig. 13). No doubt such layers are a constant characteristic of appressoria.

Passage of the germ tube through the outer wall of the epidermal cells. The germ tube produced from conidia germinating on the leaf of Bean was never found to pass through the stomata of an uninjured leaf, but always passed through the epidermal cells. It is only after the leaf has

been infected in this way at other points and the leaf cells have been in part killed, that germ tubes or hyphae pass into the stomata.

The germ tubes produced from spores sown sparsely on a leaf do not usually start penetrating the leaf immediately, but grow for a short time along the surface of the leaf and then turn down and press the point of the tube firmly on the cuticle. The germ tube is usually curved just behind the tip (Figs. 7, 17). The growing tip has a very tense appearance, being full of protoplasm which stains deeply. It is firmly anchored to the cuticle by mucilaginous material which, as described above, appears in fixed preparations as numerous threads running from the tip to the surface of the leaf (Fig. 6).

The first indication of penetration is a slight indentation of the cuticle and outer epidermal wall which can be observed where the germ tube presses closely upon the cuticle (Figs. 8, 10, 15, and 16). The next step is the actual penetration of the cuticle. The germ tube does not usually break through as a whole, but, if the epidermal wall has not been affected by the presence of neighbouring hyphae, a narrow projection is commonly put out which passes through the cuticle and enters the subcuticular layers of the epidermal wall (Figs. 14, 17, 19). A very close examination was made of these stages, but neither before nor after penetration did the appearance or the staining reactions of the cuticle give any evidence of its being softened or swollen or in any way altered chemically. *There thus seems no doubt that the cuticle is ruptured mechanically by the pressure of the tip of the germ tube.*

After penetration of the cuticle the intruding germ tube either grows straight on and enters the cavity of an epidermal cell (Fig. 21), or else it proceeds to grow more or less horizontally beneath the cuticle (Fig. 18). In either case the subcuticular layers of the wall usually soon swell up; but in a few cases, as in Fig. 21, penetration appears to have occurred without such swelling of the subcuticular layers.

No swelling of the subcuticular cellulose layer was observed before the passage of the invading hypha through the cuticle. This is in agreement with the results obtained in the third of these studies (Brown, 2) when it was shown that the active extract of *B. cinerea* had no effect on the underlying tissues when placed upon the surface of so sensitive a structure as a rose petal, though its effect was marked directly the continuity of the cuticle was broken.

When once the subcuticular layer of the walls has swollen, hyphae which enter later usually grow horizontally in this layer. Such a direction of growth is no doubt the path of least resistance, and the layer itself doubtless supplies the fungus with suitable nutriment.

It is to be noted that in the cases described above the germ tube enters without the development of any appressorium. Many cases, however, can

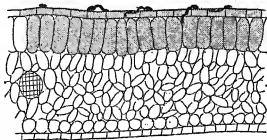
be observed where penetration of the cuticle is delayed to a somewhat later stage. In these cases the tip of the germ tube or hypha swells up and spreads out on the surface of the leaf (Figs. 9, 11, 14) and in some cases subsidiary swellings may be produced (Figs. 19, 20). These swellings are, of course, of the nature of simple appressoria. From the swollen hypha where it is closely applied to the leaf a peg-like hyphal outgrowth now appears which pierces the cuticle and pushes its way into the wall of the epidermal cell (Figs. 14, 19). Here also there is no sign of any softening or other chemical action on the cuticle as a careful study of preparations treated with scharlach red shows; there can be no doubt that, as in the case just described, the perforation of the cuticle is due solely to mechanical action, i. e. to the pressure exerted by the peg-like outgrowth. For the exercise of such pressure the hypha from which the outgrowth proceeds must be held firmly to the cuticle; this is achieved by a mucilaginous investment of the apex of the germ tube or of the appressorium. Apart from this adhesion to the cuticle due to the mucilaginous investment, the germ tube is always firmly pressed against the cuticle. Such a pressure is no doubt brought about by an extension in length of the germ tube, which is, at the same time, fixed at its basal end; the basal end, in the case of a young germ tube like that of Figs. 6, 7, may be practically the spore.

Brown (1) in the first of these studies shows that there is no evidence for the secretion by *B. cinerea* of a special toxic substance other than a cell-wall-dissolving enzyme. This is fully borne out by our observations, for in no case were the epidermal cells seen to be adversely affected in any way before the penetration of the cuticle by the germ tubes. We are quite unable to confirm the observations of Nordhausen (7), who described the killing of epidermal cells below the infection drop even when the conidia were only in the very early stages of germination.

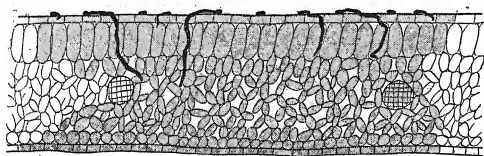
Even after penetration of the cuticle has occurred the first obvious change in the epidermal cells is usually the swelling of the walls, while disorganization of the protoplast follows later. It is clear that the hypothesis of a crystalloidal toxin which can diffuse through the uninjured cuticle and kills the cells beneath, as assumed by Nordhausen (7) and Smith (9), is quite untenable. These results are fully supported by Brown's study of the physiological conditions in the 'infection drop' published in the same number of this journal (Brown, 2).

Changes produced after penetration. As already stated, immediately after the cuticle is penetrated and the hyphal ingrowth reaches the subcuticular layer of the wall, this layer begins to swell up. As the swelling gradually increases this layer becomes laminate in structure (Fig. 19). The swelling may be so great that the lumen of the epidermal cell below may be almost completely obliterated.

The swelling of the cellulose subcuticular layer appears to stretch the cuticle. Possibly this facilitates the entry of other hyphae which find less resistance in the 'thinned out' cuticle, for in the drop infections the number of hyphae entering increases rapidly after the first few have penetrated the cuticle and caused the swelling of the subcuticular layer of the wall. The softening of the cellulose layers which underlie the cuticle



TEXT-FIG. 1.



TEXT-FIG. 2.

For explanations see p. 396.

would also markedly reduce the resistance which that membrane could offer to perforation by mechanical pressure.

When once a number of hyphae have entered the leaf other hyphae are seen to enter through the stomatal apertures, though first infection of the leaf was never observed to be brought about in this way. How far this is due to the filling of the intercellular spaces of the leaf with fluid, either from the liquid on the leaf or by the cell sap exuding from dead cells, is still uncertain.

The question also as to how far the penetration of the leaf is due to chemotropic stimuli arising from slight exudations of substances through the cuticle, or how far it is due to a contact stimulus, still requires further investigation.

As the hyphae penetrate through the epidermis, the cells of the palisade parenchyma become affected.¹ First, the nuclei move upwards towards the epidermis (Fig. 22), then gradually they begin to disintegrate, the chloroplasts swell, and starch almost disappears from the affected region. The

¹ There was no clear evidence of the movement of the nuclei of the epidermal cells towards the outer walls as a response to the development of the fungus on the leaf in the manner described by Ritter (7). The nuclei of the epidermal cells of *normal* leaves are sometimes found in close proximity to the outer wall.

dark coloration, which is one of the characteristic signs of death in the bean leaf, gradually spreads through the leaf. Text-fig. 1 is a diagram of a leaf in which the epidermal cells only are infected, but one hypha has grown down and come in contact with two palisade parenchyma cells. All the palisade parenchyma cells below the infected epidermal area are however discoloured, thus showing that the death of these cells has taken place in advance of the fungus. Text-fig. 2 shows a leaf in which the fungus has penetrated to the spongy parenchyma cells, but the discoloration of the tissues has spread through to the lower epidermis. This discoloration may not be solely the result of the enzyme diffusing out in advance of the invading hyphae, but may be partly due to action of substances liberated from the dead or dying cells.

The action of *Botrytis* on the epidermal and parenchyma cells respectively is somewhat different. In the epidermal cells the walls usually swell before the protoplast shows much alteration. In the mesophyll, on the contrary, the first morbid change is seen as slight disorganization of the protoplast; the swelling of the wall is not noticeable till a later stage. It is of course possible that changes occur first in the cell wall but are overlooked owing to its thinness.

SUMMARY.

The early stages of infection by *Botrytis cinerea* of the leaf of the broad bean (*Vicia Faba*) have been studied. The spores were grown in drops of turnip juice on the leaf.

When the germ tube produced from a spore has reached a length of about one spore-diameter it can be shown to possess a mucilaginous investment. By means of this sheath it becomes firmly fixed to the substratum.

The germ tube exerts a considerable pressure on the underlying leaf tissues, as is shown by the slight depression of the epidermal wall below it.

Actual penetration of the leaf is usually brought about by the development of a fine peg-like outgrowth from that part of the germ tube which is firmly pressed against the leaf surface.

Penetration can occur without the development of an appressorium.

Prior to the penetration of the cuticle no softening, nor swelling, nor any other change can be observed in the cuticle itself or in the underlying layers of the epidermal wall. The piercing of the cuticle is due solely to the mechanical pressure exerted by the germ tube as a whole or by the special outgrowth from it. Such a method of penetration would clearly be impossible in the absence of the mucilage which holds the germ tube firmly in position and enables it to exert the appropriate pressure.

As soon as the germ tube has forced the cuticular barrier enzyme action can occur, as is shown by the swelling of the subcuticular layers.

Death of the epidermal cells in advance of the penetration of the cuticle by the germ tube was never found to occur. Even after penetration the walls of the epidermal cells usually swell prior to the disorganization of the protoplast. There is thus no microscopic evidence for the secretion by *B. cinerea* of a special toxic substance other than the cell-wall-dissolving enzyme.

The results of a microscopical study of the early stages of infection of a leaf by the germ tube of *Botrytis cinerea* are thus in full agreement with the purely physiological observations of Brown (1 and 2).

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EXPLANATION OF FIGURES IN PLATE X.

Illustrating Prof. V. H. Blackman and Miss Welsford's paper on *Infection by Botrytis cinerea*.

The host tissue in every case is that of the leaf of *Vicia faba*.

Fig. 1. Germinating spore. $\times 1,200$. (Leitz water-immersion objective.)

Fig. 2. Germinating spore. $\times 1,200$. (Leitz water-immersion objective.)

Fig. 3. Germinating spore: older stage showing a slight mucilaginous sheath. $\times 1,200$. (Leitz water-immersion objective.)

Fig. 4. Germinating spore: older spore showing thick mucilaginous sheath. $\times 1,200$. (Leitz water-immersion objective.)

Fig. 5. Germinating spore mounted in a colloidal solution of silver; the mucilage sheath is clearly visible. $\times 1,200$. (Leitz water-immersion objective.)

Fig. 6. Young germinating spore attached to epidermis of a leaf which has been already infected elsewhere; the epidermal wall has swollen owing to this infection. $\times 1,200$.

Fig. 7. Young germinating spore anchored to the leaf surface. $\times 1,200$.

Fig. 8. Group of germinating spores; one germ tube is exerting sufficient pressure to push the wall inwards. $\times 1,200$.

Fig. 9. Young germinating spore: the germ tube has begun to spread out on the epidermis to form an appressorium; the wall of the germ tube is slightly altered at the place of contact. $\times 1,200$.

Fig. 10. Young germinating spore lying on cuticle and pressing it slightly inwards. $\times 1,200$.

Fig. 11. Young hypha swelling out at end to form an appressorium. $\times 1,200$.

Fig. 12. Appressorium forming at the end of a hypha. The wall at the apex of each branch is slightly modified. $\times 1,200$.

Fig. 13. Young appressorium showing mucilaginous sheath. Drawn from fresh material. $\times 1,200$.

Fig. 14. Young plant of *Botrytis* with a very small appressorium; the outgrowth has just passed through the cuticle of a previously infected leaf. $\times 1,200$.

Fig. 15. Tip of hypha pressing wall of epidermal cell inwards. $\times 1,200$.

Fig. 16. Ditto. $\times 1,200$.

Fig. 17. Two young *Botrytis* plants which are surrounded by shrunken remains of dehydrated mucilage. One plant has pushed through the cuticle by a small peg and is spreading out in the subcuticular layer; the second plant is already growing in the swollen wall. $\times 1,200$.

Fig. 18. A hypha which is growing in the subcuticular layer of the epidermal wall. $\times 1,200$.

Fig. 19. A group of appressoria. The pore by which the hypha has entered the subcuticular wall is shown. In this case the wall was specially stained to show the laminate structure of the swollen wall. Other hyphae have already entered the leaf. $\times 1,200$.

Fig. 20. Group of appressoria; the small pore through which entrance is effected is shown. In this case the hypha has gone straight through the epidermal cell. $\times 1,200$.

Fig. 21. A hypha which has penetrated the leaf by means of a small pore; it has continued to grow downwards through the cell, but has caused no swelling of the wall. $\times 1,200$.

Fig. 22. A portion of a transverse section of a leaf. A hypha has penetrated the cuticle and is growing in the outer epidermal wall; the nucleus of the cell has an amorphous appearance. The nuclei of the two cells immediately below have moved upwards in response to a stimulus caused by the intruding hypha. $\times 450$.

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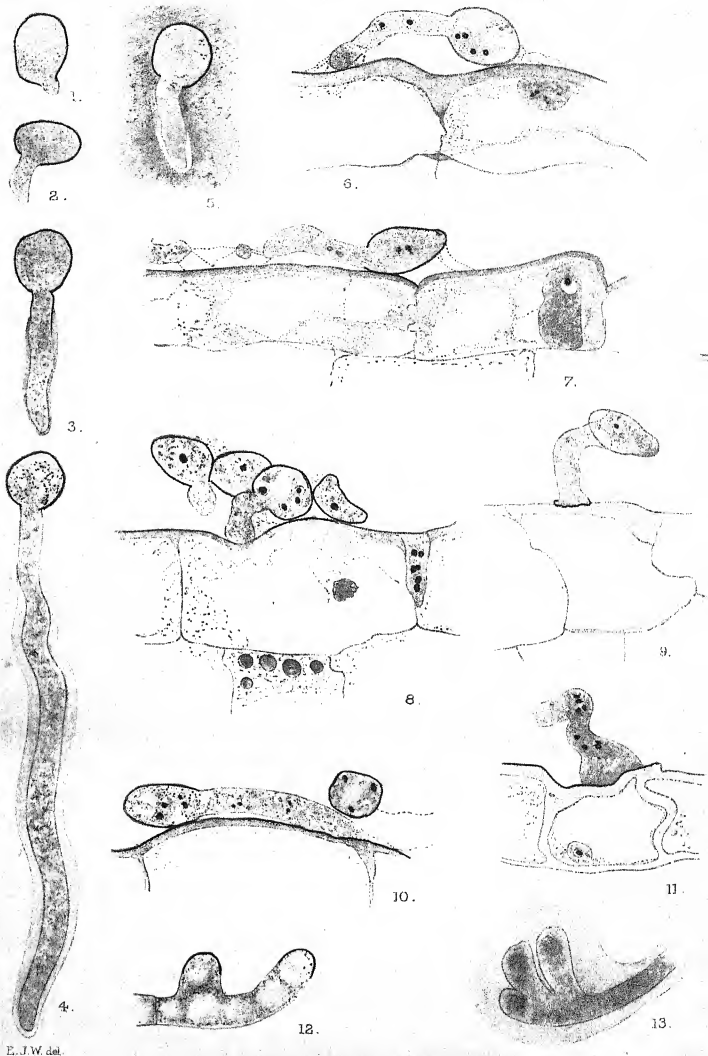
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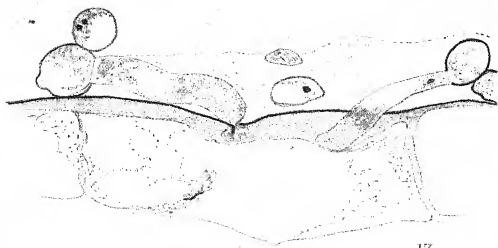
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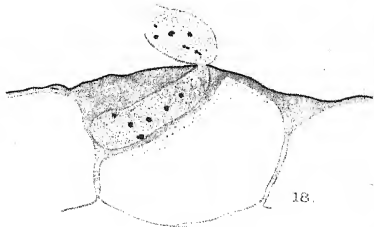
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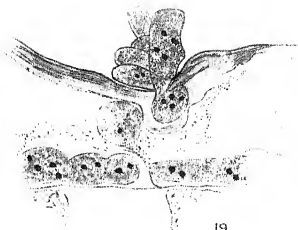
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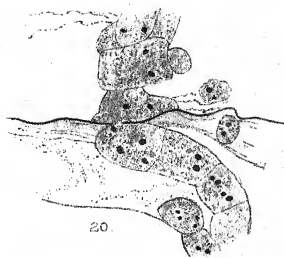
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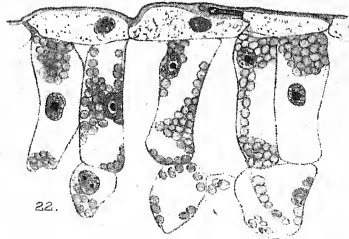
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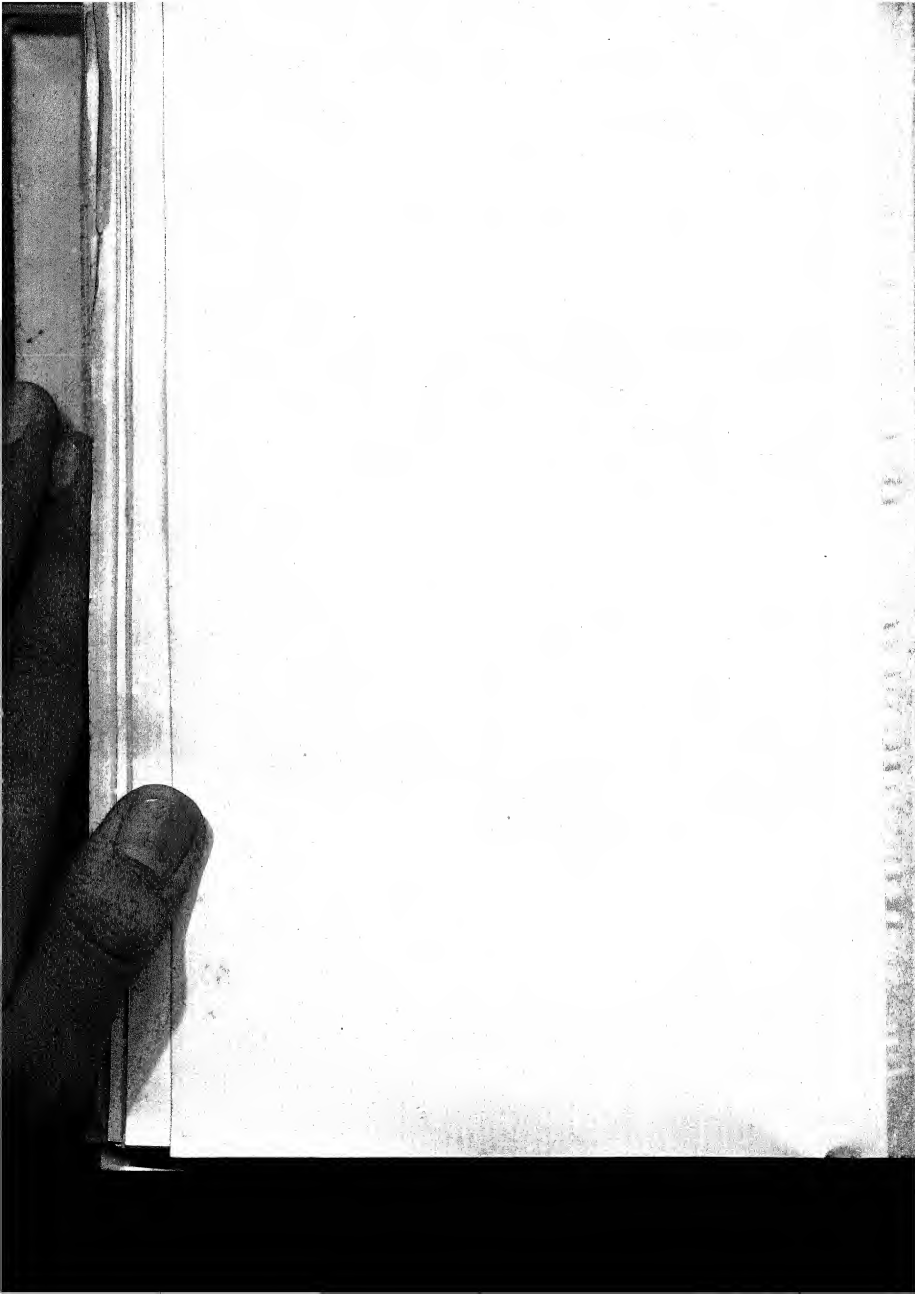


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Studies in the Physiology of Parasitism.¹

III. On the Relation between the 'Infection Drop' and the underlying Host Tissue.

BY

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IN the first of these studies, when the general action of the extract of *Botrytis cinerea* was examined, it was found that the cuticle appeared to offer a great obstacle to the action of the extract, so that in order to obtain rapid results it was necessary to inject the extract into the tissues. The object of the present paper is to examine the relation of the extract to cuticle, and more generally to determine the relation of the 'infection drop'² to the underlying tissue of the host plant. This problem is of considerable biological interest, as on its solution will depend to a large extent our conception of the physiology of the early stages of parasitic attack by fungi of the type of *Botrytis*.

The only work which deals directly with this question is that of de Bary and of Nordhausen. De Bary³ examined the early stages of the attack by *Sclerotinia Libertiana* on the stems of broad bean. Placing the bean stems a short distance in front of the advancing hyphae, he was able to arrange that the latter came in contact with the surface of the stem after a short passage through the air; the stem was thus in contact with the aerial portion only of the mycelium. Under these circumstances, the hyphae did not immediately enter the tissue but proceeded to form attachment organs. While these were being formed de Bary noted that the underlying cells collapsed and became blackened; and this at a time when, as he states, the fungus had not yet penetrated the cuticle of the plant. As a consequence of the death of the underlying cells an exosmosis of nutrient material took place on to the surface. The fungus,

¹ For No. I of this series see Ann. Bot., vol. xxix, 1915, p. 313; for No. II, see Ann. Bot., vol. xxx, 1916, p. 389.

² By this term is meant the drop of fluid in which the spores lie upon the surface of the plant.

³ de Bary, A.: Ueber einige Sclerotinien und Sclerotienkrankheiten. Bot. Zeit., 1886.

now invigorated, commenced an active growth, sending out hyphae in all directions, and in particular through the cuticle of the bean stem into the internal tissue. When the fungus was applied to the bean stem in a drop of nutrient material, de Bary found a similar sequence of phenomena, with the exception that the formation of attachment organs was omitted. De Bary's argument on this subject is as follows: 'The cause of the omission of attachment organ formation (in the latter case) can hardly be other than that the hyphae which are directly surrounded with nutrient secrete the toxin necessary for the softening (*Erweichung*) of the substrate more rapidly than those which have grown through the air, and to which nourishment must first be drawn from the distant assimilating mycelium; that therefore the softening of the host tissue which makes penetration possible takes place immediately after contact of the fungus with the epidermis, so that the resistance which constitutes the stimulus for the formation of attachment organs is not forthcoming.'

On the basis of these observations, de Bary postulates a toxic substance which is capable of diffusing through the cuticle. In examining his extracts from this point of view he was unable to arrive at any definite conclusion. In some cases he found that the fungal extract when placed on the epidermis had no action whatever on the underlying tissue; in others he found that some action did take place. Action of the extract through the cuticle was as a rule more definite with the liquid obtained from sclerotia than with the extracts obtained from ordinary mycelium. The failure to obtain positive results in all cases was ascribed by de Bary to the extract losing its activity before it could get through the cuticle.

Nordhausen¹ accepted de Bary's conclusions as a working hypothesis in his experiments on *Botrytis*. He states that while it is difficult to obtain infection when the spores are sown in large drops of liquid, infection is readily produced when the drops are small. Failure of infection when the drops are large he considers to be the result of undue dilution of the toxic substance, which therefore fails to reach a sufficiently high concentration to be effective. This effective concentration, on the other hand, is readily attained when sowings are made in small drops. As a result the underlying cells are killed and infection is thereby assured. Two experiments of Nordhausen may be specially mentioned:

1. In order to see if the fungus in process of penetrating a membrane excreted any acid substance (such as oxalic acid), he stained pieces of epidermis of *Allium* with Congo red, and allowed the fungus to grow through these. Any excretion of acid would be shown by formation of the blue acid dye in the neighbourhood of the penetrating hyphae. No such effect was however observed.

¹ Nordhausen, M.: Beiträge zur Biologie parasitärer Pilze. Jahrb. f. wiss. Bot., vol. xxxiii, 1899.

2. In order to determine if the parasitic action of the germinating spores was diminished by removing the toxic substances formed in the infection drop, Nordhausen experimented as follows: A strip of filter paper was placed in the infection drop and a current of water made to flow through the paper. Such an arrangement might be expected to carry away a large amount of the toxic substances formed by the drop. Nevertheless the entrance of the fungus was not in any way prejudiced. From this experiment Nordhausen concluded that the toxic substance must be very active even in a state of extreme dilution.

It may be observed, in passing, that the conclusions drawn from the experiment just described are in direct contradiction with those drawn from his observations on the relative efficiency of large and small infection drops.

In the light of the above work it was important to determine whether the extract employed throughout the present investigation was able to penetrate from the outside into the tissue of the host plant. In carrying out this series of experiments, two methods of applying the active extract were adopted:

1. In one series the extract was applied in considerable quantities contained in a chamber formed by a glass ring which was attached by vaseline to the surface of the leaf; the chamber so formed had a capacity of 1-1.5 c.c. This method lent itself readily to the investigation of the activity of the extract at the end of the experiment, so that it could be seen how far failure of the extract to penetrate the cuticle could be set down to its loss of activity by lapse of time, as suggested by de Bary.

2. As the amount of available air might conceivably be concerned in the process, a series of experiments were carried out in which the extract was applied to the leaf in the form of drops comparable in size with the average infection drop (about 30 drops to the c.c.).

The extract was tested on wounded as well as on intact leaves, the wound being produced by shaving off a small piece of epidermis. Also the active extract was compared with extract which had been de-activated by heating to 65° C. The tests were repeated many times and on the leaves and flowers of a large number of plants.

In the case of the wounded petals and of the great majority of wounded leaves a distinct action on the part of the extract spreading outwards from the region of the wound could be seen (in a few hours in the case of petals, overnight in the case of leaves). Where no action could readily be demonstrated under these conditions, the experiments on the corresponding uninjured leaves were rejected. It was certain therefore that all the unwounded leaves and petals under consideration would show a readily noticeable reaction to the presence of the extract if any passage of the latter through the cuticle took place.

In the case of the majority of plants examined, passage of the active

principle of the extract through the cuticle was not observed to take place. Such a negative result was invariably found in experiments with leaves and petals (both surfaces) of *Viola*, *Petunia*, *Dahlia*, leaves of *Vicia Faba*, *Begonia heracleifolia*, &c. In a number of cases a certain amount of variation in the results obtained was noticed. Thus, with some specimens, no entrance whatever took place; with others of the same species a certain amount of action was observed. This took the form of discoloured spots, varying in number and extent, but in no case did anything approximating to a general discoloration spreading over the surface of contact make its appearance. Such discoloured spots were occasionally obtained in tests on petals of *Tropaeolum* and *Geranium*; and more frequently in the case of petals of *Rosa* and *Fuchsia*. Nevertheless, even in rose petals which showed many discoloured spots after twenty-four hours' treatment with the extract, portions could be found which were quite unaltered.

Bearing in mind the possibility of the existence of previously undetected injuries of the cuticle (such as insect bites, &c.)¹ as well as the possibility of accidental contamination with living spores which is not always readily detected, we may state that there is no action on the part of the fungal extract when applied to the cuticle; in other words the cuticle, apart from accidents which may occur in cases with some frequency, constitutes an impassable barrier to the passage of the active principle of the fungus. The experiments with petals of *Viola*, which are readily attacked by the fungus, show, moreover, that the germ tubes can easily infect a tissue the cuticle of which is completely impervious to the fungal extract.

In the preceding experiments the extracts employed were such that on injection into the various tissues the latter were disintegrated in the striking manner described in the first paper of this series. Furthermore, there was no question of the extract having lost its activity during the period of the experiment. One particular experiment may be cited in illustration of this point.

A fungal extract when laid on a rose petal for twenty-four hours produced no change whatever. After this lapse of time, injection of the same liquid into the same petal produced complete disintegration within half an hour.

Finally, in a parallel series of experiments with infection drops consisting of a dilute suspension of spores in a dilute nutrient, discoloration was found to take place in 12-24 hours from the time of sowing. An investigation shortly to be published has shown that at no time during the period from sowing to discoloration can the concentration of active

¹ Such injuries may occur very frequently, even in apparently healthy leaves. See in this connexion a paper by Barker and Gimingham, *Annals of Applied Biology*, vol. 1, 1914, p. 13.

principle in the infection drop at all approximate to its concentration in the extracts employed in these experiments.

The above results are contrary to the view of de Bary according to which killing takes place previous to penetration of the cuticle by the fungus. The discrepancy can only be explained by assuming either that the conclusions of de Bary do not hold for *Botrytis cinerea*, or that the method of extraction here employed has failed to obtain some toxic substance which plays an important part in the early phases of natural infection. All question of the presence of a cuticle-dissolving enzyme may be put on one side, as no evidence has yet been obtained that any fungus produces such an enzyme; and certainly no such enzyme is present in the extract in question.

For the application of de Bary's result to the case of *Botrytis* attack, there is no direct evidence apart from Nordhausen's statement that browning and killing of the cells of a moss leaf took place before the hypha had penetrated and even before the spore had germinated. This statement has been criticized in another place,¹ and it is very doubtful if any weight can be attached to it in this matter. Again, the experiment with stained epidermis of *Allium* discounts the excretion by the fungus of any acid substance, at any rate of such a comparatively strong acid as oxalic acid. The experiment in which the toxic products of the fungus were removed or considerably diluted by means of a stream of water can only be readily interpreted according to the view that there is in the infection drop no toxin which is capable of passing through the cuticle. The incompatibility with one another of certain of Nordhausen's conclusions renders further criticism of his work unnecessary.

The hypothesis of killing in advance of penetration of the cuticle necessitates a crystalloidal toxin. The maximum concentration of this toxin would occur in the neighbourhood of the hypha; but owing to the high diffusibility which must be postulated of the toxic substance, it is impossible to believe that any considerable concentration gradient of toxin could subsist within the limits of the infection drop. A toxic substance capable of diffusing through the cuticle on the one side would freely diffuse into the spore-free region of the infection drop on the other. The action of such a toxin should therefore not be confined to the immediate neighbourhood of the hyphae, and the existence of such a substance should also be demonstrable in the general liquid of the infection drop.

The remainder of this paper is devoted to the examination of the infection drop from the point of view of the presence of a crystalloidal toxin and in particular of a soluble oxalate.

¹ No. I of this series. *Ann. Bot.*, vol. xxix, 1915, pp. 329, 330.

A. *On the question of the presence of a crystalloidal toxin in the infection drop.*

The following observations have been made :

1. In many cases the first signs of discoloration of the tissue underlying the infection drop appear along the margins of the latter. This effect is best seen when the drops do not spread over a large surface of the leaf. Microscopic observation shows this effect to be correlated with more rapid germination of the spores along the margin. The fact that such a variation in the time of attack can be obtained within the limits of a drop so small as 0.03 of a c.c. cannot be explained on the basis of the de Bary hypothesis.

2. If a drop containing spores be placed on the leaf, the spores allowed to settle into contact with the epidermis (a process which requires about one hour), and the drop be now slightly displaced so as to include a new portion of the leaf surface, the discoloration as it first appears, and for a considerable time afterwards, is seen to outline strictly the area originally occupied by the drop; in other words, the discoloration effect is strictly localized to the immediate neighbourhood of the germinating spores. This result is again directly contrary to the hypothesis of de Bary.

3. With the same number of spores, the size of the drop can be varied within wide limits without appreciably affecting the time of appearance of discoloration; and similarly with drops of the same medium and of the same size the number of spores can be varied within wide limits without producing any marked variation in the time of appearance of discoloration. Thus sowings on rose petals in which the concentrations of spore suspension were in the ratio 10 : 1 gave 10–12 hours and 12–14 hours respectively as the times at which discoloration could be first detected by the naked eye. If the discoloration effect were due to the accumulation of a toxic substance in the drop, the effective concentration should be reached much more rapidly in the case of the denser sowing (the concentration of nutrient in the drop being sufficient to allow of vigorous germination), and the time of appearance of discoloration should therefore be correspondingly accelerated. Nevertheless we find that the shortening of the period between sowing and the appearance of discoloration with increasing concentration of spores is very slight. Bearing in mind the fact that discoloration in its earliest stages is not apparent to the naked eye, and further that when once started, the degree of discoloration would be proportional roughly to the number of spores present, we can readily conceive that the slight time difference above noted is explicable in this manner.

4. When infection drops, in which discoloration was just becoming visible, were collected, cleared of spores by centrifuging, and tested on the surface of the most sensitive petals, no toxic action whatever could be demonstrated.

In all the preceding experiments, the spores were sown in a medium of sufficient strength to enable germination of all the spores present to take place. It may be thought that in such a case there might be no killing antecedent to penetration, there being sufficient nutriment already present in the infection drop; but that the formation of the toxin might be called forth by a condition of starvation in the infection drop. This hypothesis is *a priori* improbable; the following experiments were made to settle this point:

Suspensions of spores of equal density were made in water and in a series of media of strength $m, \frac{m}{2}, \frac{m}{4}, \frac{m}{8}, \frac{m}{16}, \frac{m}{32}, \frac{m}{64}$, where m represents a glucose-peptone medium (glucose 1 per cent., peptone 0.2 per cent.). Within this range of strength of medium all variations were obtained from very poor to fairly vigorous germination. Drops of these suspensions were placed on petals of rose and on leaves of broad bean. In all cases discoloration was produced, but in the case of sowings in water on bean leaf only very slowly. When discoloration was established over the surface of contact of the original drop with the leaf, the drop was slightly displaced so as to include a small portion of the adjacent surface. In no case, however, was any discoloration of the newly included surface shown.

The above experiments show conclusively that there is no accumulation in the infection drop of a substance which can diffuse through the cuticle and bring about death of the underlying cells.

B. On the presence of a soluble oxalate in the infection drop.

The evidence already brought forward under (A) applies also to this special case. As considerable importance has been attached by various writers¹ to the part played by oxalates in conditioning parasitic attack, it may not be considered superfluous to bring forward certain additional evidence on this point.

In the infection drop, as has already been stated, the first appearance of discoloration of the underlying tissue (rose petal, bean leaf) was observable in about twelve hours from sowing. Now in parallel experiments it was determined that, in the case of both plants, a concentration $\frac{n}{40}$ of oxalic acid when placed in the form of drops on the surface produced no noticeable action within a period of twelve hours. The same statement applies to a concentration $\frac{n}{20}$ of potassium oxalate. If oxalic acid or oxalate be the toxic substance concerned, the concentration of the same in the infection drop must at least equal $\frac{n}{40}$ or $\frac{n}{20}$ in the respective cases. In

¹ See especially R. E. Smith: Bot. Gaz., vol. xxxiii, 1910, p. 385.

the infection drops employed there was however no possibility of such high concentrations, either of acid or of salt. In the experiments in this connexion a glucose-peptone medium was employed. This medium contained a trace of a calcium salt (derived from the commercial peptone preparation), which in the medium as made up was determined by titration to be of strength

$$\frac{n}{800} - \frac{n}{1,000}.$$

Sowings were made in this medium in the usual way, and as soon as discoloration was observed the drops were collected and cleared of spores by centrifuging. The addition of a drop of potassium oxalate to this liquid gave a distinct precipitate, thus showing that a soluble calcium salt was still present in the infection drop. It is plain therefore that the figure $\frac{m}{800}$ represents an upper limit to the amount of oxalate excreted by the fungus into the infection drop. The actual amount of oxalate formation, if any, is certainly much below this figure. Thus in plate sowings of two days' age in which the same glucose-peptone medium was employed, the excretion of soluble oxalate had not gone so far as to precipitate all the calcium from solution.

It appears therefore that the formation of soluble oxalate in young fresh cultures of *Botrytis*, if it take place at all, is negligible in amount, and that vigorous attack by the fungus can take place under conditions where the presence of a soluble oxalate is quite excluded.

CONCLUSIONS.

The infecting germ tubes of *Botrytis cinerea* are unable to affect chemically the cuticle of the host, nor do they secrete any toxic substance which can pass through the cuticle and bring about the death of the underlying cells. The fungus is unable to affect the underlying tissue until the obstacle afforded by the cuticle has been overcome. Chemical action being excluded, penetration of the cuticle must take place in a purely mechanical way. Once penetration of the cuticle has been accomplished, the underlying tissue is attacked after the manner described in the first number of this series.

The results of this purely physiological study are in exact agreement with the results of a microscopical study by Blackman and Welsford¹ of infection of the bean leaf by *Botrytis cinerea*.

¹ No. II of these studies. Ann. Bot., vol. xxx, 1916, p. 389.

Roesleria pallida, Sacc.¹

BY

JESSIE S. BAYLISS-ELLIOTT, D.Sc.,

AND

W. B. GROVE, M.A.

With eleven Figures in the Text.

AT the end of the year 1915 we obtained from Sutton Coldfield a number of specimens of a fungus which was considered at first sight to be *Pilacre Petersii*; they were growing on the roots of a willow (Fig. 1) which had died at the end of autumn, after passing through a period of



FIG. 1. Ascophores of *Roesleria pallida* on the roots of Willow (nat. size).

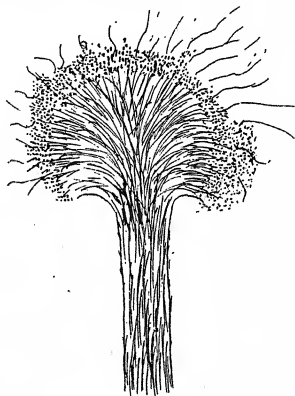


FIG. 2. Vertical median (microtome) section of an ascophore, showing the hemispherical hymenial disc. $\times 120$.

gradual exhaustion during the preceding summer. It is a natural inference that the fungus was the cause of death. On closer examination the spores were seen to be in asci, and the fungus was obviously a species of *Roesleria*

¹ Mich. ii. 299.

(Fig. 3), but on attempting to discover which species it was we encountered an unexpected difficulty: it did not agree with any of those described. We had met with a similar fungus before on roots of Apple and Beech, but in both cases it had been put down without sufficient examination as *Pilacre*.

The question then arose whether it was a new species of *Roesleria* or not. By the courtesy of the authorities of Kew, we have been enabled to examine their specimens of *Roesleria* and *Pilacre*, viz. *R. pallida*, *R. pilacriformis*, *P. faginea*, and *P. Petersii*. We find all the specimens

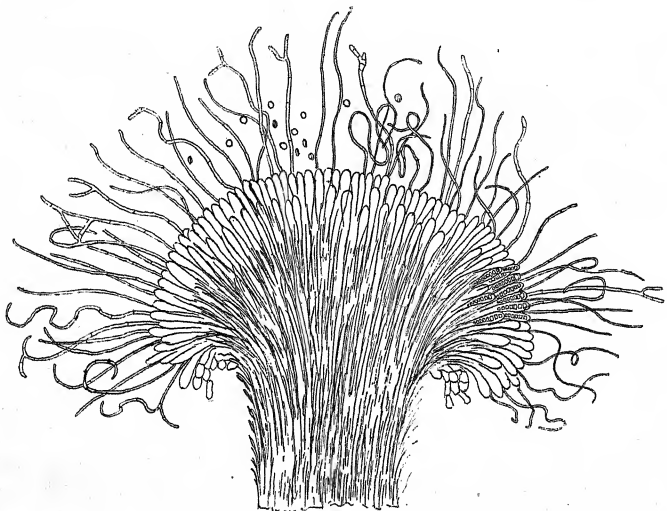


FIG. 3. Section through ascophore of *R. pallida*, after the older paraphyses forming the peridium have been brushed away. $\times 300$.

under the first two names to be exactly alike, and to agree perfectly with the fungus which we had in hand except in one small detail (the darker colour of the stems), but all of them differed from the descriptions of Saccardo, Rehm, Massee, Prillieux, Richon, &c., in some respect or other. The conclusion at which we have arrived by this inquiry is that all the three are *R. pallida*, but that the descriptions of that species require an important modification.

Moreover, it became evident that it would do no violence to the facts if

it were concluded that *Pilacre faginea* and *P. Petersii* were also identical with each other, and that both resembled the *Roesleria* so much in character as to make it seem not unlikely that *Pilacre* is only a stage of *Roesleria*. Experiments with a view to test this latter point are being set on foot, and we wish at present merely to record our belief in the truth of that statement.

Again, it was not possible to find any confirmation of Brefeld's contention that *Pilacre* is a Protobasidiomycete: nothing could be seen resembling his drawings of basidia (Untersuch., 1888, Heft vii, Pl. I). The spores grew exactly as he represents them, but uniform 'basidia' were not present, merely branches of the conidiophore (Fig. 10). The conclusion at which we have arrived is that *Pilacre* is a conidiophorous fungus, not in any sense a Basidiomycete, and that it is not in the remotest degree allied to the Auriculariaceae and Tremellineae, but is a stage of the Discomycetous genus *Roesleria*. They differ chiefly, as regards external appearance, in the usually stouter and shorter stem and larger head of *Pilacre*. It is well known that to give the latter name at first sight to a *Roesleria* is not at all an uncommon incident even among expert mycologists.¹

The form and stature are similar in all these four species of *Pilacre* and *Roesleria*: the mode of occurrence is the same, the texture of the head and of the stem are the same, the manner of production of the spores between the convex upper surface of the head and a pseudo-peridium is also the same, the sole difference being in the exact form and colour of the spores, and their origin in asci or not. There are, of course, many Discomycetes which are known to have conidial stages, although no close parallel can be cited to the combination here advocated.

The description of our specimens is as follows:

ROESLERIA PALLIDA.

Apothecia at first greyish, somewhat pruinose, fawn-coloured when old, gregarious or in little clusters, 1-4 mm. high (including the stem, which is whitish, becoming yellowish, then darker, $\frac{1}{4}$ - $\frac{1}{2}$ mm. thick): head varying in diameter, sometimes not much wider than the stem, at others up to 1 mm. wide. Asci subcylindrical when young, cylindrical when mature, 28-32 \times 5-6 μ (part sporifer.), on a short pedicel, 8-spored. Spores monostichous,

¹ *Pilacre Friesii* and *P. subterranea* Weinmann have proved to be ascophorous and = *R. pallida*. No doubt these occurrences are due to the great elusiveness of the asci, a good view of which we find can only be obtained by brushing away the free ascospores and the tangle of paraphyses, lying loosely over the hymenial disc, before cutting or teasing it for microscopical examination. Even in microtome sections of material carefully fixed and prepared, the asci do not stand out so clearly as when treated in the way indicated above. Whether the asci can be seen or not, however, the two form-genera can be distinguished with ease at once by the shape and colour of the spores: there is now no reason why they should ever be confused.

round and compressed, circular in face view, $4\frac{1}{2}$ – 6μ broad, narrowly elliptical in profile, $2\frac{1}{2}$ – 3μ thick. Paraphyses very much longer than the asci, filiform when young, then branched and anastomosing, septate, whitish, 3μ broad, undulated, and entangled at the periphery so as to form a pseudo-peridium. Asci soon diffuent, and spores then forming a thick layer between the hymenial disc and the peridium.

The spores are singly colourless, but yellowish or isabelline in mass. They are placed in the ascus with their flat sides in contact, like a pile of coins (Figs. 4 and 6). Each of them is lens-shaped in vertical section, the

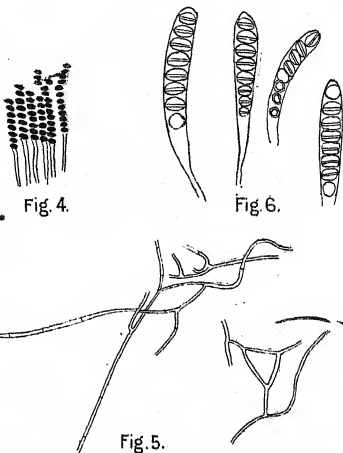


FIG. 4. Portion of hymenium, showing ascospores (stained), apparently in 'chaplet'-form on a pedicel. $\times 400$. FIG. 5. Anastomosing paraphyses forming the peridium. $\times 400$. FIG. 6. Asci containing ascospores. $\times 800$.

margin of the lens being slightly flattened so as to form a ridge surrounding it like a frame: this causes the spore when seen in face view to be bordered with a narrow halo (Fig. 8, *a*), and is the origin of the phrase 'crasse 1-nucleatis' applied to them by Saccardo. When seen in exact profile, the spore appears to be crossed along its greatest diameter by one or two narrow dark lines (according to the focusing of the objective), which are the places where the surface undergoes a sudden change of convexity (Fig. 8, *b*). When the spores are in the young ascus they are tightly packed so as to give them a squarish appearance (Fig. 7), but when the ascus begins to

break up they are seen like a 'chaplet' or chain of eight conidia perched upon a little stalk (Fig. 4).

The figure of Rehm (Rabenh. Krypt.-Flor., vol. i, pt. 3, p. 385) shows in a sectional view of the apothecium a decided cup which does not exist at all in any of the specimens which we have examined; nor is the hymenium flat as there represented, but convex and hemispherical, or even umbilicate beneath (Fig. 2). The asci are not distinctly clavate, nor are the spores distichous. The spores in the intact ascus (except sometimes the end ones) never show their widest diameter like a chain of spherical conidia, as represented by Rehm, but only after they have been disturbed. The mistaken idea about the spores, which are described by Rehm as 'kuglig-rund', arose from the fact that under the microscope the majority of the loose spores naturally settle on their flat sides, and look quite round. The edge-wise lying spores were either overlooked or ignored. The old idea mentioned by Saccardo (Syll. Fung., iv, 579), that *Roesleria* was a genus closely allied to *Pilacre* (both being Hyphomycetes), arose from the excessive diffidence of the asci. The spores can often be seen, still in rows, but without any sign of the ascus (Fig. 4); these chains then break up and finally form a dense pulverulent mass interspersed among the radiating paraphyses. The enormous quantity of the spores is due to the fact that the asci are produced and ripen successively over a long period. In a fresh undisturbed state the outside of the mass of spores is bordered by the projecting upper ends of the paraphyses, which are sometimes colourless and at others look somewhat brownish.

Massee's figure (Diseases of Cult. Pl., p. 289) is also incorrect, as it shows the spores as if perfectly spherical and arranged in the same manner as in the figure of Rehm.

Rehm's *Coniocybe pilacriformis* (l.c., p. 1223), which is *Roesleria pilacriformis*, Hennings, on roots of Rose and *Paliurus*, is exactly identical with some of the slender forms of *R. pallida*. The stalk of the latter often closely approaches 'reh-braun', and is less than $\frac{1}{4}$ mm. thick, as Rehm describes his species; his spores are 'monostichous, 6-7 μ long and broad, or 5 μ broad', which again can be paralleled among some of our specimens, since they are in fact a little nearer, on the whole, to *R. pilacriformis* than to *R. pallida*.

R. hypogaea (= *R. pallida*) is described by Prillieux (Bull. Soc. Bot. Fr., 1881, xxviii, 275) and by Gillet as occurring in quantity on the roots of vines, causing the disease called 'Pourridié des Vignes'. It has also been found in this country on the roots of Rose-trees, and abroad on the bark of Oak, Lime, Elm, fruit trees, Poplar, Maple, Alder, and Hornbeam.

About *Pilacre*, it was found that authentic specimens (from Berkeley's herbarium) of *P. Petersii*, B. and C., are perfectly identical with *P. faginea*, B. and Br., as was stated by Tulasne so long ago as 1865 (Ann. Sci. Nat., Bot.,

5^e sér., iv. 294). The blackish stem attributed to the latter is merely the result of age. It is true that many of the specimens of *P. Petersii* have a relatively larger and whiter head than some of *P. faginea*, but also specimens of which exactly the opposite may be said are not uncommon. No other specific distinction worthy of notice is even alleged, unless it be the more flexuous conidiophorous hyphae of *P. faginea*, and that is not evident in all the

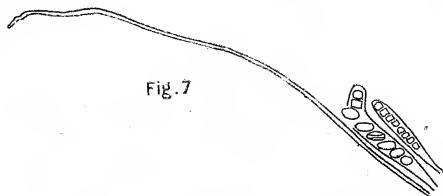


Fig. 7



Fig. 8.



Fig. 9.

FIG. 7. A young ascus, an ascus dehiscing, and a young paraphysis. $\times 800$. FIG. 8. Spores: *a*, seen lying on their flattened surface; *b*, seen in profile. $\times 800$. FIG. 9.—A very young ascophore, no asci present, only paraphyses. $\times 90$.

specimens. The spores are exactly the same in both, being yellowish-brown (snuff-coloured in mass) and bun-shaped, that is, convex above and flattened or even slightly umbilicate beneath, where there is often to be seen the remains of the very short sterigma still attached (Figs. 10 and 11). Corda's figures of the spores of his *Botryochaete faginea* (Icon. vi, f. 95) agree with Brefeld's figures of his conidial stage of *P. Petersii*, at any rate in their older state, for the apiculus at the base of Brefeld's conidia is only distinct

when they are young; the umbilicate base appears to us to arise only when the spores are perfectly mature and ready to fall off.

The sole difficulty in the way of this identification is that Corda figures (l.c.) swollen 'basidia' bearing 1-4 transversely placed spores, but he figures these on the same hyphae as others which look like spherical conidia, although he does not himself differentiate them in his description; while Brefeld's 'basidia' are elongated and transversely septate, but the spores are identical in shape, pose, and colour. No signs of any such structures were seen by us, except in a very indefinite and evidently casual way. Nevertheless, *P. faginea* abounded in the very same clamp-connections (Fig. 10) and short lateral branches growing beside them (Brefeld's

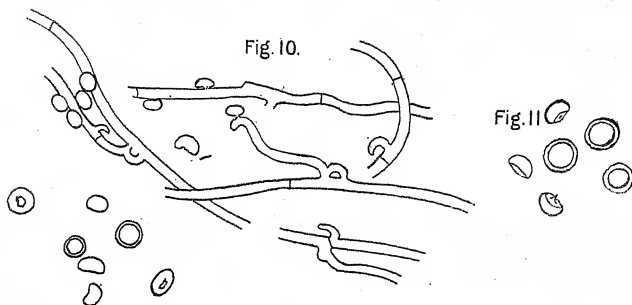


FIG. 10. *Pilacre faginea*. A portion of the hyphae forming the head, showing the 'buckle' arrangements, just as figured by Brefeld for *Pilacre Petersii*, and also conidia attached and free. $\times 800$. FIG. 11. *Pilacre Petersii*. Spores in face-view and profile. $\times 1,000$.

'basidia'), exactly as figured by him in his *P. Petersii*. Both kinds of 'basidia' appear to us to have little or nothing to do with the real basidia of the Basidiomycetes.

Stilbum pilacriforme, Richon (Bull. Soc. Bot. Fr., 1882, xxix. 241), which may not differ very much from a species of *Pilacre*, was considered by its author to be the conidial stage of *R. hypogaea*, occurring several months before the ascophorous stage appeared. It seems likely also that several species of *Pilacre* described by Berkeley, *P. tephrospora*, *P. orientalis*, *P. depressa* (not to mention others) may well be nothing but forms of *P. faginea*, but no specimens of these have been examined. It is not impossible that the whole of the species mentioned in this account should be included under the one title, *R. pallida*, Sacc. There remain as probably distinct species *R. hyalinella*, *R. candida*, and *R. crocata*, for *R. onygenoides*,

Karst., seems to be nothing but another form of *R. pallida*, judging from its description. These are, however, merely suggestions of probabilities.

In choosing between *Pilacre* and *Roesleria* as a name for the asco-phorous genus, it is reasonable to hold that so long as the first described species of the former genus, *P. Weinmanni* (Fr. Syst. Myc., 1829, iii. 204) remains unidentified, it is better to retain that generic name for the conidio-phorous Fungi such as *P. Weinmanni* appears to be, and use *Roesleria* (Thüm. and Pass., 1877) for the ascophore, while *Coniocybe* should be reserved for the lichenologists. Should it, however, be finally proved that *Pilacre* and *Roesleria* are stages of the same fungus, the former name being the earlier would perhaps be chosen. The name *Ecchyna* of Fries (Summa, 1849, p. 446, note) and Patouillard becomes then superfluous.

In conclusion, we wish to tender our thanks to the authorities at Kew, to Professor G. S. West, and to Mr. J. Ramsbottom of the British Museum, for assistance in this investigation.

SUMMARY.

1. *Roesleria pilacrisformis*, Henn. is only a slender form of *R. pallida*, Sacc.
2. *Pilacre Petersii* is identical in every respect with *P. faginea*.
3. Both species of *Pilacre* are not basidiophorous, but purely conidio-phorous Fungi, and have not the remotest connexion with the Auriculariaceae and the Tremellineae.
4. *Pilacre* is probably a conidial stage of species of *Roesleria*.

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Conjugate Nuclei in the Ascomycetes.

BY

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With four Figures in the Text.

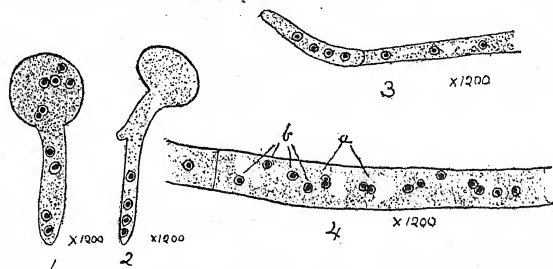
DURING the last few months an investigation of the process of infection by *Botrytis cinerea* has revealed the fact that the nuclei in the multinucleate hyphae of this fungus, produced by the germinating conidia, are very generally arranged in pairs. Such hyphae were very well nourished, having developed at a temperature of 26°C. either in strong turnip juice or in the tissues of the leaf of *Vicia Faba*. Mycelia from conidia grown in water or much-diluted turnip juice (1 in 16) did not show paired nuclei, neither were these found in the narrow ill-nourished hyphae which occasionally occur in the parasitic mycelium in the Bean leaf. The paired condition of the nuclei is only found in well-nourished fast-growing filaments, and hence it seems to be correlated with rapid growth. The pairing can be explained by the fact that nuclear divisions follow one another so rapidly that the sister nuclei formed by one division have not time to move apart to any considerable distance before they start dividing again.

Fig. 1 is a drawing of a conidium growing in strong turnip juice; all the nuclei are in the paired condition; the members of the pairs in the germ tube are farther apart than those in the conidium; the separation is no doubt preparatory to division. An older stage is shown in Fig. 4; only part of the hypha is drawn; it had entered its host plant and was thriving on nourishment obtained from the epidermal cells. The pairs of nuclei marked *a* have recently divided, those marked *b* have no doubt moved apart in preparation for further division. The fact that an unpaired nucleus is rare in a well-nourished filament indicates that the sister nuclei divide simultaneously. A striking contrast to these well-nourished hyphae is shown in Figs. 2 and 3. Fig. 2 represents a germinating conidium twenty-four hours after sowing in a drop of distilled water on a glass plate; the germ tube which can only draw nourishment from the conidium has very few

nuclei, and the conjugate character is not well marked. As the reserve food of the conidium is used up the paired condition becomes still less evident. Fig. 3 shows an older and more starved hypha grown in *very dilute* turnip juice; in the second cell no indication can be seen of the nuclei being in pairs.

A similar phenomenon has been found in the mycelium of *Sclerotinia Libertiana*. The nuclei in well-nourished hyphae growing in the stem of *Vicia Faba* are arranged in pairs, whilst in starved hyphae the nuclei are unpaired.

As pointed out by Fraser¹ in 1913, conjugate divisions are not at all uncommon in the different parts of the Ascomycetes. They have been observed by various writers: in the conidial mycelium of *Hypomyces perniciusum* (Massee); in the germ tubes of the ascospores of *Ceratostoma*



FIGS. 1-4. Conjugate nuclei in *Botrytis cinerea*. All figs. $\times 1200$.

brevirostra (Nichols); in the paraphyses of *Helvella crispa* (Carruthers); in the large storage cells of *Helvella elastica* (McCubbin); and in the ascogenous hyphae of a large number of Ascomycetes (Maire, Guillermond, Claussen, Faull, &c.). The presence of conjugate nuclei in the ascogenous hyphae has been very frequently noticed, and it has been regarded by certain authors (Guillermond, &c.) as a special sexual phenomenon. Both Claussen² and Faull³ lay great emphasis on this arrangement of the nuclei in the ascogenous hyphae, regarding the synkarion which they find there as consisting of a male and a female nucleus. Faull asserts that the 'conjugate

¹ Fraser, H. C. I. (Gwynne Vaughan): Development of the Ascocarp of *Lachnea Cretea*. Ann. Bot., xxvii, 1913, p. 559. Full references are given in this paper.

² Claussen, P.: Zur Entwicklungsgeschichte des Ascomyceten *Pyronema confluens*. Zeit. f. Bot., iv, 1912, p. 1.

³ Faull, J. H.: The Cytology of *Laboulbenia chaetophora* and *L. Gyrodiumum*. Ann. Bot., xvi, 1912, p. 325.

divisions play an important part' in the life cycle of the plant, and considers that 'cognizance of the phenomenon' must be taken in all investigations into the sexuality and phylogeny of the Ascomycetes.

If, however, the results here described are shown to be general for the Ascomycetes, and there is some evidence of this, the observation of conjugate nuclei in the ascogenous hyphae would give us no help in elucidating the sexual phenomena of the group. The paired condition of the nuclei may simply be the response to the physiological conditions usually found in such hyphae, that of high nutrition associated with rapid growth.

The Development of 'Sanio's Bars' in *Pinus Inops*.

BY

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With four Figures in the Text.

THE 'bars of Sanio' are the short rods or bars found stretching, in most cases, from the two tangential walls of the tracheides, cambial cells, and phloem elements, in many Conifers and protruding as small rod-like projections into the lumen of the tracheides in the veins of the leaves of *Juniperus*. In many cases the bars in the wood of Conifers occur as a series of radial rods extending throughout several annual rings. In the xylem region these bars have lignified walls, and in the cambium and phloem walls of cellulose.

The history of their investigation dates back to Sanio (1), and reference to them is occasionally found in the works of later investigators down to the present time. Sanio in his work records what he termed at first a false cell-wall crossing the cell cavities of the tracheides in *Hippophae rhamnoides* and in *Pinus sylvestris*. In radial section these appear as rods crossing from the inner to outer walls, and in tangential section as spots on the tracheide walls. Sanio regarded these at first as possibly being the end walls of tracheides, but abandoned this idea later. He further stated that in radial sections of wood the bars sometimes appear as little sprouts on each tangential wall of the tracheides. Sanio's suggestion as to the origin of the bars is that they arise by a bending in of the two tangential walls of the tracheides, but he failed to find a corresponding depression of the outer contour of the tracheide, and further suggested that this may be due to some optical effect caused by some chemical alteration of the tracheide wall at that point. It was Sanio who first demonstrated that in the xylem region the walls are lignified and in other regions cellulose.¹

Independent of Sanio, von Mohl (2), on investigating the leaf structure of *Sciadopitys*, found bars present in the vascular elements, and further

¹ Confusion was introduced as to these bars by Miss Gerry (Ann. Bot., 1902), by reference to the clear areas above and below the bordered pits of coniferous wood as being the 'bars of Sanio', and further by stating these bars were of a cellulose nature.

records bars occurring in the xylem of the leaves of *Juniperus communis*, *J. Oxycedrus*, *J. oblonga*, and *J. macrospora*, but in this case mostly occurring as projections which end blindly in the lumen of the tracheide.

About the same time C. Winkler (3) recorded the occurrence of bars in *Araucaria brasiliensis*.

The following year Russow (4) observed Sanio's bars in the wood of tracheides of *Abies Pichta* and *Pinus sylvestris*, and found them crossing ten successive annual rings and passing through the cambium and young bast elements: he further records their presence in *Pinus nigra*, *P. Pinca*, *P. Strobus*, *P. Laricio*, and *P. anachuite*.

Mention is also made of rod-like outgrowths by de Bary (5), under the head of 'Tracheides with Transverse Bars', who records them as occupying the corners of the vascular bundles of the stems of *Lycopodium* and in the margins of the vascular bundles of *Juniperus* leaves. de Bary stated that they are somewhat flattened, cylindrical rods branching irregularly on all sides, the branches fusing with one another to form a network through the cavity in some cases, and in others forming thickenings on the walls of the tracheides. In the leaves of *Juniperus* their points of attachment and origin are especially the margins of the bordered pits, in *Lycopodium* the margins of the spiral or reticulate thickenings of the lateral walls. In *Biota orientalis* in the vascular bundles of the leaves the swollen margins of the bordered pits are often elongated into blunt cones, which protrude into the cavity, but end blindly without coalescing with one another or with the opposite wall. With these facts before us, the projections found on the inner walls of the ray tracheides of *Pinus* might also be compared with Sanio's bars, for they project into the lumen of the tracheides, as do the ingrowths in the leaves of *Juniperus*.

In 1890 C. Müller (6) pointed out the wide distribution of these bars, and he gave them the name of 'Sanio's bars', indicating that they occur in the four groups of Coniferae (Abietineae, Cupressineae, Podocarpeae, and Taxineae). Müller further indicated their possible mode of development, and discusses three possible modes of origin as follows:

1. The bars are secretion products of special protoplasmic accumulations in the cambium cells.
2. The bars are produced by partial reabsorption of the transverse walls of tracheides.
3. The bars are formed by an infolding of the tangential walls of the tracheides.

In support of (1) he compared the formation of a cell-wall in cell-division where the new wall is formed from the protoplasm, the formation of a cell-wall in a *Vaucheria* filament when injured in any way, and the formation of cell pegs or bars in *Caulerpa*. His objection to (1) is that if the bars are produced in this way it is not easy to see why they should not

fill the whole lumen of the tracheide, i. e. be a complete transverse wall; and it is difficult to explain their occurrence in radial rows at the same height in the cambium cell, as the protoplasm is constantly streaming. The strand of protoplasm would therefore be stationary, and further the vertical radial plate-like nature of the bars is not possible on this hypothesis. Müller alleges that the bar form, stretching from wall to wall, is not shown in the cambium cell; the bars are free at both ends. (As will be shown later, this statement is not correct.)

2. In support of Müller's second possible mode of origin, that the bars are produced by partial reabsorption of the transverse tracheide walls, he stated that the presence of square ends to tracheides is not uncommon in *Ginkgo*: in some cases rows of tracheides occur, each tracheide having a square end wall. Against this view, however, Müller points out that square end walls are rather the exception than the rule, and that a partial solution of transverse walls is otherwise not known in coniferous wood; and he further points out that if reabsorption took place in tangential sections the bars would appear elongated in the transverse direction, whereas they are elongated in the long direction of the tracheide.

3. In support of his third suggestion, that the bars are formed by folding of the tangential tracheide walls, he points out the infolding of the cell-walls in the mesophyll of Pine leaves, also the folding of the walls of transfusion tissue and the occasional folding over of a tracheide when it comes in contact with a medullary ray.

The view finally adopted by Müller is:

'Sanio's bars' arise from folds of the radial walls of the cambium cells, and the transition from plate to bar form depends on partial reabsorption, which results in the setting free of the bar as a consequence of the total reabsorption of the bar groundwork in the cambium cell.

In 1892 W. Raatz (7), working independently at the same time as Müller, published at a later date his observations on 'Sanio's bars', and added to the list of species in which the bars occur, including the xylem of the veins of the leaves of *Hippophae rhamnoides*, root, stem, and secondary wood of *Salix fragilis*, and *Casuarina equisetifolia*. Raatz pointed out that the bars are not universal phenomena and not structures to be relied on as always occurring, and further stated that in *Abies pectinata* frequently little projections occur on the middle of the bar.

Raatz summarizes the development of the bars in the following way:

1. The bars are more or less abnormalities, not arising in any regular order and not having any definite function.

2. The bars are formed by the tangential walls of the cambium cell coming in contact, and as this cambium cell gives rise to a tracheide and the walls become lignified, so the walls of this bar becomes lignified.

From the various theories as to the origin of 'Sanio's bars' and the

confusion arising therefrom it appeared desirable to reinvestigate their development. Specimens from about twenty-five species of *Pinus* were obtained in May, 1914, and fixed in watery picric acid.

Amongst the species collected was *Pinus Inops*, which, on examination, showed the bars most frequent, and so appeared most suitable for investigation.

Transverse, radial, and tangential sections were taken; for transverse and radial hand sections were found most suitable, but for tangential the

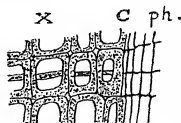


FIG. 1. Transverse section of *Pinus Inops*, showing 'Sanio's bars' crossing xylem (x), cambium (c), and phloem (ph). $\times 520$.

microtome was used after embedding the wood in paraffin wax of 52° C. melting-point.

In transverse sections the bars were found to cross the lumina of the tracheides as relatively thick lignified rods, and in the cambium to be merely fine thread-like structures with cellulose walls, these threads sometimes widening out again in the bast or retaining their thread-like character, but still with cellulose walls. The way in which the bars cross the cambium and phloem could be more easily seen in radial than transverse sections, where, in addition to crossing the sieve tubes, the thread-like bars could be demonstrated crossing the phloem parenchyma (Figs. 1 and 2).

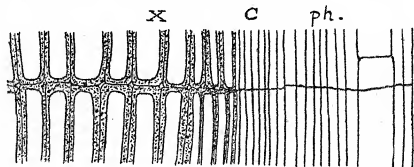


FIG. 2. Radial section of *Pinus Inops*, showing bar of Sanio crossing xylem (x), cambium (c), and phloem (ph). $\times 415$.

The most interesting part of the investigation came out in the serial microtome-cut tangential sections, where whole series of sections could be obtained, tracing the bar outwards from the xylem through the cambium and into the phloem, the bar appearing in section throughout.

In the xylem region the bar appears as a hollow tube varying in out-

line from circular to oval, sometimes the opening being obliterated owing to its lateral walls coming together (Fig. 3, D to F).

An attempt was made to find out whether any substance was present in the cavity of the bar; protoplasm, cellulose, and fungal hyphae stains

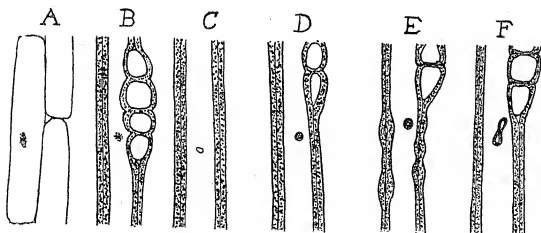


FIG. 3. Series of tangential sections passing from cambium through the tracheides, showing change of shape and variations of thickness in the bar whilst passing through various parts A-F. $\times 390$. A, in cambial region; B and C, in tracheides near cambium; D-F, in successively more remote tracheides.

were used respectively, but in no case was it found possible to demonstrate any substance present.

Tracing the bar outwards from a distance of two or three tracheides from the cambium, it was noticeable that, as the neighbourhood of the cambium was reached, the bar became slender with a smaller lumen and

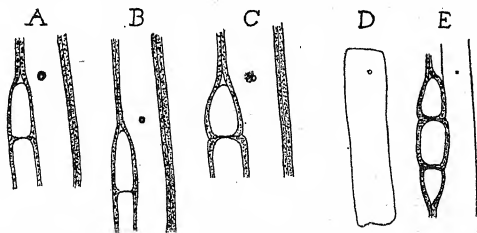


FIG. 4. Series of tangential sections, showing bar in section in cambium and through the two tracheides nearest to cambium. $\times 390$. A and B, in xylem elements; C, D, E, in cambial elements.

thinner walls, and frequently in the tracheides nearest to the cambium small masses of protoplasm were often found attached to the bar laterally (Fig. 3, B; Fig. 4, C).

Passing through the cambium with the bar in section, the bar becomes reduced to a small dot showing no hollow in its centre; if a hollow is present

G g

it is too small to demonstrate. Frequently this dot-like structure is completely enveloped in protoplasm (Fig. 3, A and B), or has small masses of protoplasm attached to it laterally. The thread-like bar continues through the cambium radially and often widens out again in the phloem region.

In all these tangential sections the bar rarely occurs near the transverse end walls of a tracheide, but most frequently about half-way between the end walls. From the evidence obtained by the presence of protoplasmic masses around the bars in the cambium region, it would appear that once started in *P. Inops* the bars continue their existence by means of these protoplasmic masses, but exactly what starts them could not be demonstrated. That they are formed by partial reabsorption of the end walls of tracheides, as suggested by Raatz and Müller, does not seem to hold for this species, as the end walls of the tracheides where they are flattened frequently have bars present a short distance above or below this end wall. The view put forth that these bars are formed by tangential walls of the cambium cells bending in, until they come in contact, did not appear to be the case in *P. Inops*, as throughout the whole radial width of the cambial cells they did not vary in diameter, which would be the case if a folding of the wall took place, as they would appear smallest in diameter in the middle of the cell and gradually increasing towards the outer or inner tangential walls. The following would appear to be the probable explanation of the observations recorded in this paper:

In its first stage as yet revealed a 'Sanio's bar' is a very delicate cylindrical rod stretching radially and freely across a cambium cell in a transverse plane. As the cambium cell grows in a radial direction, the rod keeps pace with it by stretching along its main axis. The cambium cell eventually cuts off a daughter-cell, which in turn grows radially and directly or indirectly, after a further division, gives rise to a tracheide (or sieve tube), with whose radial increase of width the bar keeps pace. At the same time surface growth of the tangential walls of the growing daughter-cell or cells takes place in longitudinal transverse and intermediate directions. The bar, firmly fixed into the primary walls, is at its ends exposed to tension at right angles to its long axis in all directions, and the natural result is a rupture in the centre of the rod, which now becomes hollow. At first it would appear that there is no disparity in rate of growth in surface of the tangential walls in the longitudinal and transverse directions, so that the hollow in the centre of the axis itself is cylindrical in form; but when, as is the case later, growth in length of the cell greatly predominates, the rod is exposed at its ends mainly to tensions at right angles to its main axis in the radial longitudinal plane of the cambium cell, with the consequence that the bar and its central cavity are stretched in a corresponding manner, and the appearance in cross-section is that shown in Fig. 3, F. The bar is thus from a very early stage a solid elastic body, since the tension in

a direction at right angles to its main axis is transmitted from the two ends to the middle, so that the rod becomes hollow along its whole length.

At the same time the rod becomes thicker by means of deposits on it of layers of wall-substance (which in tracheides assumes the form of lignified material and in phloem cellulose). Such a deposit recalls the mode of centripetal thickening of pegs and the like projecting in from the walls of *Caulerpa*, and the projections in the mesophyll of Pine needles and other plants, also the deposit of cellulose on hyphae of a parasitic fungus traversing a living cell whose protoplasm is responsible for the cellulose in question. This explanation explains the continuity of the Sanio bars throughout successive tracheides and sieve tubes, the growth in thickness, the origin of the central hollow, and the final shape of the mature bar, but there is no clue to the original inception of the bar in the cambium cell.

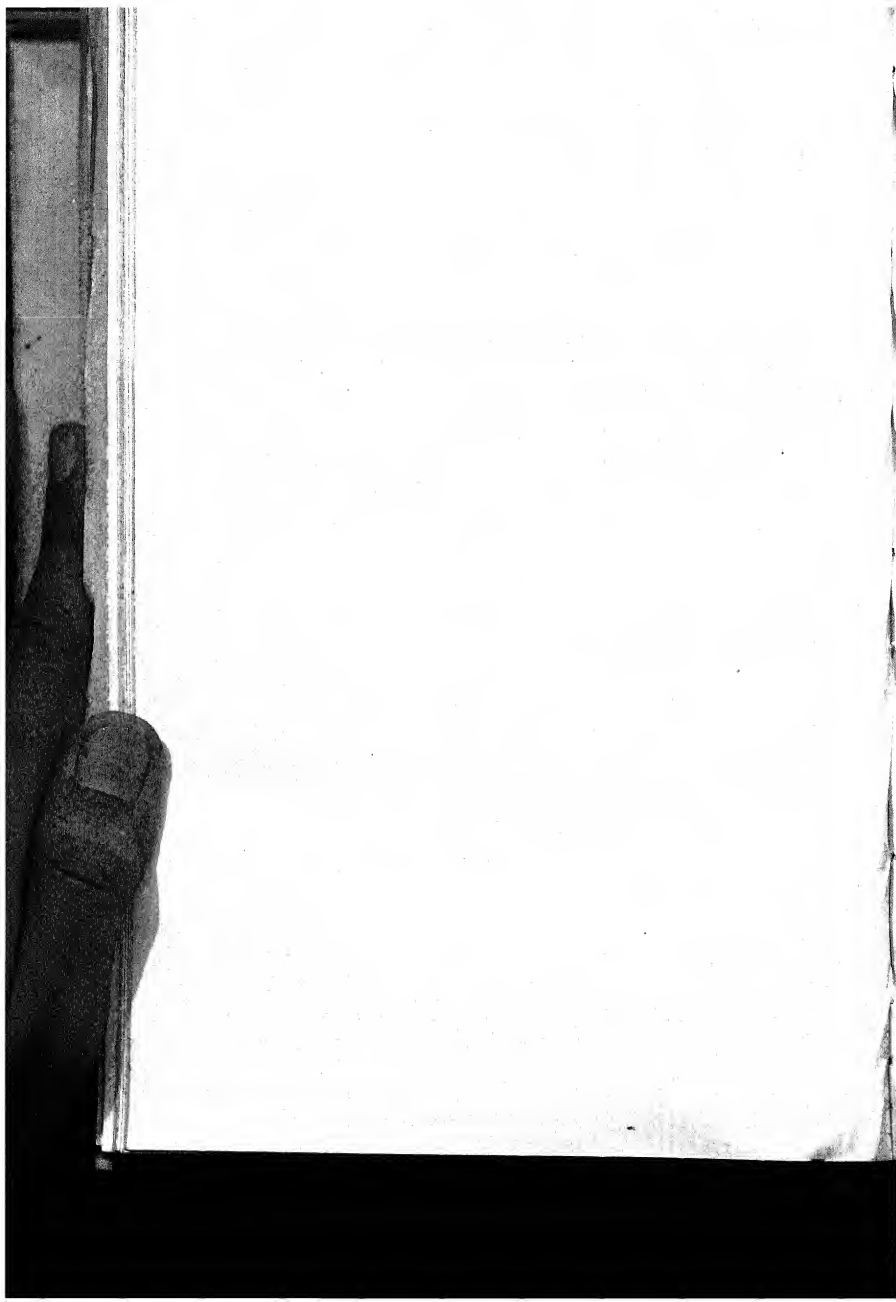
SUMMARY.

1. Sanio's bars are small rods crossing the tracheides, cambium, and phloem elements in many Conifers.
2. In phloem and cambium the walls are of cellulose, whilst in the xylem region they are lignified.
3. In cambium the bars exist as thin solid rods, and in the xylem and phloem as more or less hollow rods.
4. Small masses of protoplasm frequently surround the rods in the cambium cells and suggest a possible mode of origin.

In conclusion, I wish to convey my best thanks to Professor Percy Groom, of the Imperial College, for his help and profitable suggestions during the progress of the investigation, and to Mr. A. W. Hill, Assistant Director of the Royal Botanic Gardens, Kew, for allowing me to have specimens of Pine woods from the Kew Collection.

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On the Interpretation of the Results of Water Culture Experiments.

BY

WALTER STILES.

INTRODUCTION.

WHEREVER a process is a function of several independent factors, it is necessary to consider the possible influence of all the others when the relation between the rate of the process and any one of the factors is examined. In plant physiology the outstanding example of this is to be found in carbon assimilation. The processes summed up under this term depend upon at least three independent variables: carbon dioxide, temperature, and illumination. As a result of F. F. Blackman's exposition, it was shown that increase in any one of these factors brought about a corresponding increase in assimilation, *provided the other factors were present in quantity sufficient for the assimilation to proceed at the increased rate*. Thus to choose one factor, temperature, the rate of the assimilation is more than doubled if the temperature is increased 10° C., but it is self-evident that when the assimilation proceeds at this increased rate more than twice as much carbon dioxide will be used up in any given time than at the lower temperature. Now if in this time the leaf is not supplied with this larger quantity of carbon dioxide, the assimilation obviously cannot proceed at the maximum possible for that temperature. The amount of carbon dioxide under these conditions is a limiting factor.

In water culture experiments there is usually measured the increase of growth, during a certain time, corresponding to some definite composition of the nutrient solution. In other words, experiments with water cultures attempt to determine the effect upon growth of factors acting through the root system. As growth depends upon so many factors, it is very necessary in drawing conclusions from water culture experiments to bear in mind the possible action of factors other than the one under investigation. That the neglect of this is probably the cause of divergent results obtained by different experimenters with the water culture method will be shown in this paper.

THE COMPLEXITY OF THE SYSTEM.

Growth, in the sense of increase in the total dry matter of a single plant, is the resultant of a large number of inter-related processes, of which

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the chief may be considered as assimilation and respiration. Any factor which will influence either of these will also influence growth. Assimilation in the wide sense may be dependent upon all those environmental factors that influence carbon assimilation, chief among which are temperature, carbon dioxide, and light. In addition it may be influenced and therefore limited by factors acting in the first place through the root system, water-supply, and supply of nutrient substances in the solution surrounding the roots. The respiration will certainly be influenced by the concentration of oxygen and carbon dioxide in the solution surrounding the roots, and the concentration of these gases in the nutrient solution will thus influence growth.

Besides these obvious factors, others are possible. For instance, the concentration of the nutrient solution might influence growth by affecting the absorption of necessary salts, or by influencing transpiration. It has been shown by the writer¹ that under certain conditions the concentration of the nutrient solution over a fairly wide range of variation does not act as a limiting factor in growth in water culture. Recently Dr. W. Brenchley² has asserted the contrary, her conclusions being based on several series of experiments in which the growth of plants was apparently dependent on the concentration. It will be shown here that Dr. Brenchley's results are in all probability due to the confusion of two possible factors—the absolute amount of salt available, and the concentration of the solution; and that in her experiments the growth was limited, not by the concentration but by the insufficiency of the total quantity of salt supplied, the initial concentration of the solutions not being maintained throughout her experiments.

THE FACTORS.

In discussing the various factors which may influence the rate of growth of plants in water culture, only those will be considered which act through the root, as it is for the purpose of investigating these that water culture experiments are usually carried out. At the same time it must be recognized that factors acting through the subaerial part of the plant may limit growth, and that generally during the twenty-four hours of any day a series of factors probably limits growth at different times. Only a very approximate parallelism can then be expected between the rate of growth and any particular factor even when this is the chief limiting factor during the day.

1. *Supply of 'Nutrient' Salts.* As the plant body consists of complex substances which contain various elements only derivable from salts present in the solution surrounding the roots, it is clear that if the

¹ Stiles, W.: On the relation between the Concentration of the Nutrient Solution and the Rate of Growth of Plants in Water Culture. *Ann. of Bot.*, vol. xxix, 1915, pp. 89-96.

² Brenchley, W. E.: The Effect of the Concentration of the Nutrient Solution on the Growth of Barley and Wheat in Water Cultures. *Ann. of Bot.*, vol. xxx, 1916, pp. 77-90.

supply of any one of such salts should be below a certain amount, such a salt may act as a limiting factor on growth. When this is the case an increase in salt supply will bring about a corresponding increase in growth. On the other hand, if some other factor is limiting growth, an increase in salt supply will not result in any increase in growth.

If through the limiting action of any factor, as for example temperature, the rate of growth is small, we may therefore expect plants to be unaffected over a wide range by the total amount of nutrient salt supplied. When, however, owing to increase in the value of this factor, a higher rate of growth is possible, we may expect the limiting action of quantity of salts supplied in a given time to be present over an increased range of concentrations.

Results already published appear to show this very clearly. Cultures were grown by the writer, and more recently by Dr. Brencley, in solutions of the same relative composition but of different concentrations ($1, \frac{1}{2}, \frac{1}{10}, \frac{1}{20}$). Considering only those cultures in which the nutrient solutions were frequently renewed, we may take for granted that in the cases where the highest concentration was employed the salt supply was not limiting.¹

When the rate of growth in the strongest solution is rapid, as in Dr. Brencley's experiments, although the growth is about the same when the total supply of salts is reduced to $\frac{1}{2}$, yet further reduction in salt supply causes a marked reduction in growth.

In the experiments of the writer the rate of growth in the highest strengths of solution was not so great as in the case of Dr. Brencley's plants, probably owing to temperature acting as a limiting factor. Here there was no marked reduction in growth until a much lower strength ($\frac{1}{20}$) of solution is reached.

Again, in the preliminary experiments of the writer made with Rye during the winter months, when growth was still slower, there was no significant lowering of growth even in the case of plants growing in solutions of $\frac{1}{20}$ the concentration of the strongest.

The following table summarizes the results of both writers:

Grammes KNO_3 supplied in highest strength of solution.	Growth (dry matter in grammes) in solutions of relative strength.				Observer.	Plant.
	1	$\frac{1}{2}$	$\frac{1}{10}$	$\frac{1}{20}$		
10.8	0.059	0.072	0.059	0.060	Stiles.	Rye.
10.8	0.628	0.622	0.555	0.471	"	Barley.
9.0	4.00	3.65	2.80	1.85	Brencley.	"
9.0	5.77	4.50	2.74	1.97	"	"
7.8	6.46	6.04	3.09	1.78	"	"

¹ This is indicated by Dr. Brencley's observation that 25 per cent. of the original supply of nitrate was still left in such a culture solution that was unchanged for eight weeks. However, in this case there was almost certainly limitation of growth due to other factors, as, for instance, accumulation of carbon dioxide in the culture solution.

If one considers the last series only, it will be observed that reduction of the supply of nutrient salts to one-fifth of the maximum supplied was accompanied by only a slight diminution in the amount of dry matter produced. With further reduction the diminution is very marked, and the total growth is fairly proportional to the quantity of nitrate supplied. It suggests strongly that the amount of salt supplied in the $\frac{1}{5}$ strength was just a little below the quantity required to allow the other factors present full play. A reduction below this critical quantity means that the salt supply is a limiting factor. Dr. Brenchley's published curves show this point very clearly.

One would not, of course, expect an exact proportionality between salt supplied and quantity of growth, having regard to the complexity of the system and the constant change, not only of other factors but also of the rate of salt supply. For the plants grow larger with time, both their absorptive and assimilatory systems increasing in size; but the quantity of salt supplied in the same time remains the same. The supply per unit of plant is therefore constantly decreasing. It is quite probable that only after the plants have reached a certain size will their demands on the salts be sufficient for the rate of salt supply to become a limiting factor. Thus Dr. Brenchley says, 'The development of the shoots in the plants growing in the different concentrations was very similar for some long time, but gradually a falling off was noticed with the two lowest ($\frac{N}{10}$, $\frac{N}{20}$), and by harvest time some indications of this appeared even with $\frac{N}{5}$ shoots'.

Again, Dr. Brenchley writes that her results indicate 'that with the lower strengths the amount of growth was strictly limited by the quantity of food supplied, and that it was impossible for the plants to reach full development with such a restricted amount'. This is exactly the point. The supply of some nutrient salt is acting as a limiting factor. But there is no indication that it is the *concentration* of the nutrient solution that is limiting. The possibility of this as a factor will be dealt with later.

2. *Respiration*. There seems to have been a strong tendency with workers on water cultures to avoid considering the undoubtedly important fact that roots respire. In this process roots no doubt absorb oxygen and evolve carbon dioxide, and these processes, one would think, must influence the rate of growth of a plant in water culture.

As regards carbon dioxide, it has long been recognized that the concentration of carbon dioxide in the medium external to plant tissue may influence its activity. In the case of water cultures the carbon dioxide produced by the respiration of the root will dissolve in the culture solution, and unless it reacts with any of the dissolved salts its concentration in the solution will gradually increase until it may act as a factor limiting

respiration. It is possible that in this accumulation of carbon dioxide in the culture solution is to be found the explanation of the less growth of plants growing in solutions which are not renewed, but which nevertheless contain enough salts for a more rapid growth. The following numbers, taken from a previous paper of the writer's, exhibit this point:

Initial concentration of food solution.	Solutions renewed.	Relative total quantity of salts supplied.	Growth.	Time of growth.
1.0	Once	2.0	0.31	April 30—June 9.
0.2	Eight times	1.8	0.62	April 28—June 6.
0.05	Eight times	0.45	0.47	April 28—June 6.

A diminution of the oxygen dissolved in the culture solution might also affect growth and act as a limiting factor. In the absence of any data it is impossible to say how far this factor may be operative. The partial pressure of each gas in the culture solution will always be tending to come into equilibrium with the partial pressure of the same gas in the atmosphere, but there is no doubt a constant lag in the bringing about of this equilibrium.

3. *Concentration of the Nutrient Solution.* The extent to which concentration of the nutrient solution can act as a limiting factor is difficult to examine, because from the moment the plant roots are put in the solution the concentration will be altering. It was in an attempt to minimize this difficulty that the writer changed the water culture solutions at frequent intervals. Even then the concentration of the nutrient solution will only remain very approximately constant when the rate of growth is slow. This was the case with the writer's experiments, in which it was shown that the variation in the concentration of the nutrient solution over a fairly wide range has little influence on the dry matter produced. In the lowest strengths of solution employed there was a slightly less rate of growth, but this might have been due to the insufficiency of one of the nutritive salts during the later days of growth.

Dr. Brenchley states in her recent paper that when plants 'are grown in water cultures under favourable conditions, the concentration of the nutrient solution, up to a comparatively high strength, has a great effect upon the rate of growth'. As a matter of fact her experiments do not support this statement. Indeed her results properly examined rather indicate the reverse. In the following table, compiled from Dr. Brenchley's results, it is shown how much better growth results in solutions of low concentration when these are frequently changed, than when they are not. The improvement in the case of higher concentrations is relatively much less. In order to show this clearly the weight of the plant in the never-changed solution is taken as unity in the case of each concentration in each series, and the weights of the corresponding plant in the once changed and frequently changed solutions are given relatively to this.

	Relative concentration of solution.			
	1	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$
Series 1.				
Never changed	1.000	1.000	1.000	1.000
Once changed	1.274	1.443	1.493	1.628
Frequently changed . . .	1.520	2.600	3.386	4.051
Series 2.				
Never changed	1.000	1.000	1.000	1.000
Once changed	1.188	1.319	1.404	1.332
Frequently changed . . .	1.352	2.548	2.936	3.747
Series 3.				
Never changed	1.000	1.000	1.000	1.000
Once changed	1.250	1.641	1.251	1.759
Frequently changed . . .	2.102	3.748	5.970	10.73

It will be observed that whereas the growth of culture in the strongest solutions was only improved from 1.4 to 2.1 times by frequently changing the solutions, in the most dilute solutions frequent renewal of the culture medium brought about from 4 to nearly 11 times as much growth. It strongly suggests that if the solutions had been changed still more frequently the rate of growth would have been still higher. The results indicate clearly that no conclusions whatever can be drawn from such observations as to the effect of varying concentration of the solution. They indicate that in the lowest strengths the salt supply was deficient and required very frequent renewals. In other words, the actual concentration soon fell below that of the initial concentration after renewal of the solution.

Again, Dr. Brenchley states that for a considerable time all her plants grew as far as the eye could judge at equal rates, but that after that time those in the diluter solutions grew less rapidly than the others. This suggests that in this later period of growth the plants are so large that the nutrient salts supplied in one lot of diluter solution are no longer sufficient for the growth that other factors would allow. The rate of growth is limited, not because of the concentration of the solution, but because the salt supply is not kept up constantly.

The difference between the rate of salt supply and the concentration of the solution supplied at definite intervals may be made clear by considering an extreme case. If an adult oak-tree were grown in a litre of a dilute nutrient solution, supposing such a thing to be possible, it is quite clear that it would not require many minutes for the exhaustion of the food supply. If the solution were renewed every four days, salt supply would still limit growth exceedingly. But if it were supplied with a litre of the same nutrient solution for every square millimetre of absorbing surface, it would take a much longer time for the food supply to be exhausted, and if the solution were renewed every four days it is conceivable that the salt supply would not limit the growth. It is obvious that in the first case it would be erroneous to ascribe limitation of growth to the concentration of the external solution. Yet this is in effect what Dr. Brenchley does.

It is clear from the writer's experiments, and it is indicated also by the

statement of Dr. Brenchley mentioned above (p. 430), that when plants are growing slowly the concentration of the nutrient solution is without influence on the rate of growth over a wide range. As the plant grows larger it will grow more rapidly because both the assimilative and absorptive systems are larger. If these two systems increase at the same rate, it should follow that if concentration of the solution is without influence on the rate of growth when the plant is small, so it should also be without influence on it when the plant is large, for although the plant will require more salts, the absorbing system will be larger and so can obtain more.

The whole question therefore resolves itself into the problem of the relationship between the rate of absorption and the concentration of the medium. It is quite conceivable that below a certain point concentration of the nutrient solution might be so low that the root could not absorb enough salts from the solution simply because of the dilution of the latter. No very definite evidence has, however, yet been obtained of this, although the researches of True and Bartlett¹ are suggestive in this regard. They indicate that this limiting concentration is very low.

4. *Toxic Action of Substances in the Nutrient Solution.* The presence of certain substances in the nutrient medium may act as a factor limiting growth. This has been shown to be the case with salts of a number of metals such as copper, zinc, and manganese. An account of such toxic action may be found in Dr. Brenchley's monograph on Inorganic Plant Poisons and Stimulants. The limiting action of such substances is no doubt due to their reaction with substances of the plant which disturb the normal reactions which make up the life processes.

5. *The Ratio between the Concentration of different Substances in the Nutrient Solution.* Numerous writers have urged as a result of water culture experiments the need for a definite ratio between the constituents of the culture solution for the production of optimum growth. Any wide departure from this ratio causes depression of growth, owing to one of the constituents, being present in excess, entering the plant too rapidly and producing a toxic action. The whole question has been summed up under the name of Antagonism, a general account of which is given in a paper by Mr. Jørgensen and the writer.²

There may be something in the idea of balanced solutions, but the evidence in favour of it will not bear analysis. All the experiments adduced in support of it are open to one or two fatal criticisms, and most of them to both. These are:

¹ True, R. H., and Bartlett, H. H.: The Exchange of Ions between the Roots of *Lupinus albus* and Culture Solutions containing one Nutrient Salt. Amer. Journ. Bot., vol. ii, 1915, pp. 255-78. The Exchange of Ions between the Roots of *Lupinus albus* and Culture Solutions containing two Nutrient Salts. Ibid., pp. 311-23.

² Stiles, W., and Jørgensen, I.: The Antagonism between Ions in the Absorption of Salts by Plants. New Phyt., vol. xiii, 1914, pp. 253-68.

(1) No account is taken of the great variation between individual plants growing in water cultures under the same conditions.

(2) In no case has a constantly renewed culture solution been employed. Thus the ratio of the various constituents was probably constantly changing throughout the experiments, and instead of being a constant factor was an unknown and varying one.

Hence the possibility of a lack of balance acting as a limiting factor can be admitted, but it cannot be claimed that such a limitation has been proved in any one case.

In the foregoing pages some of the factors which may limit the rate of growth of plants in water cultures have been dealt with, particularly those which act through the root system, as it is with these that water culture experiments, by their very nature, usually deal. In discussing these questions frequent recourse has been had to Dr. Brenchley's researches, and these have been exposed to some criticism, not because of all water culture experiments they are those which most call for criticism, but because they are those that require it least. They are, for instance, apart from the much fewer ones made by the writer, the only ones in which the limits of error and significance of differences are indicated, and in which therefore recorded differences have a true value. They have also been carried out on a generously conceived plan and under ideal conditions. Much information is to be derived from them as to limiting factors in water culture experiments. But owing in one instance to the confusion of two possible factors the conclusions as regards the limiting action of concentration do not hold.

In endeavouring to explain the difference between her results and the writer's, Dr. Brenchley ascribes the failure of concentration of the nutrient solution to influence growth in the writer's experiment to some other limiting factor (e. g. smoke pollution) which was ignored. Of course the whole point of the experiments was to show that it was some factor other than concentration which limited growth. The factor was probably largely temperature or perhaps illumination. It may have been atmospheric impurity as such, though this is not very likely, and it was certainly not any such factor as copper in the distilled water (which was 'conductivity' water from a block tin still), 'lack of cleanliness in working, growth of algae in culture bottles, admittance of light to the roots'. It would indeed be reprehensible negligence for a scientific worker to allow such factors as these to be operative.

It should also be pointed out that in dealing with such a complex as growth, it is extremely difficult to examine the action of any particular factor upon it. During the twenty-four hours a dozen different factors may limit growth at different times. Nevertheless it is possible and no doubt

often the case, where one factor is predominatingly the limiting one, to show this is so, and it is also possible to show that a factor is not the limiting one when alterations in its magnitude have no effect on growth.

THE LIMITATIONS OF THE WATER CULTURE METHOD.

It must be acknowledged at once that experiments with water cultures have in the past yielded information of first importance. Thus it was by their use that the elements necessary for plant growth were demonstrated. More recently in Dr. Brenchley's hands they have given much information as to the toxic properties of various substances. But it is necessary to bear in mind the limitations of the method. In the form in which it has hitherto been employed, the essential of the water culture method is to control the amount of substance added to the nutrient solution and to measure the growth corresponding to this. But growth is the result of such a complex of factors, scarcely any of which are completely under control and many of them not at all. An understanding of the physiology of the plant can only be brought about by careful analysis of factors carefully controlled, and by accurate quantitative measurement.

Again, owing to the variability of individual plants, the method leaves much to be desired as regards accuracy, unless large numbers of plants are dealt with and the probable error of experiment calculated. This makes the method very laborious.

Dr. Brenchley's last published results are probably as accurate as can be obtained by the method, and she has very properly calculated the probable error. The mean of each set of ten plants growing under the same conditions had a probable error range which was often about 4 per cent. of the mean value, rarely below that, and which was frequently as much as 9, 10, or even 11 per cent. of the mean. Hence for a difference between two mean values to have a reasonably certain significance in these experiments it would be necessary for the means to differ by about 10 per cent. to 15 per cent. in favourable cases, and by as much as 30 per cent. or even more in unfavourable ones.¹ This cannot be regarded as very satisfactory in comparison with the degree of accuracy which can be obtained by other physiological methods which at the same time are much less laborious. Nevertheless for the solving of some problems the method may be the only one available.

SUMMARY.

1. Growth, the resultant of a number of different processes, is dependent ultimately upon many separate factors, each one of which may limit the rate of growth.

¹ See, for example, Gray, F. W.: *A Manual of Practical Physical Chemistry*, London, 1914, chapter i.

2. Water culture experiments are usually performed in order to examine the influence of certain factors upon growth, by controlling the action of these factors acting through the root.

3. Owing to the neglect of the possible effect of other factors, and to the difficulty of controlling most of these factors in water cultures for any length of time, it may be difficult to obtain definite evidence of the relation between any one factor and the rate of growth.

4. It should be obvious as an elementary physiological principle, and it is amply borne out by the results of published experiments, that the supply of nutrient elements in the water culture solution can act as a limiting factor.

5. With this factor has been confused that of the concentration of the nutrient solution. No evidence has yet been obtained that concentration of the nutrient solution acts as a limiting factor, but it is probable that it may so act in exceedingly dilute solutions, and it probably does in very high ones.

6. The possibility of the influence of respiratory activity of the root on the culture solution has been neglected hitherto. This may play a part in limiting growth.

7. The water culture method is of very limited application to physiological problems on account of (1) the difficulty in analysing results due to the complex of factors not under control ; (2) the difficulty of controlling in some cases even the factor whose action is being investigated ; and (3) the excess of labour required to produce results which are only of a low degree of accuracy.

LONDON,
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The Distribution of Species in New Zealand.

BY

J. C. WILLIS, M.A., Sc.D.

With a Diagram in the Text.

IN two recent papers¹ I have dealt with the distribution of angiospermous species in Ceylon, and have endeavoured to show that it follows simple arithmetical rules, whether one is dealing with the endemic species or with those more widely distributed. From Trimen's figures for the local distribution of the flora, which are very full and accurate, I have shown that both 'wides' (as for brevity's sake I term the widely distributed species) and endemics are arranged in graduated series, the former being most numerous at the 'very common'² end of the scale, the latter at the 'very rare'³ end. In both cases the numbers decrease towards the other end of the scale, i. e. they decrease in opposite directions.

Not only do the grand totals show these graduated results, but each family and genus of reasonable size shows the same, and so do the smaller genera and families when added together into small groups. From this fact of the exactly similar behaviour of all groups I have drawn the conclusion that natural selection cannot be responsible for the actual distribution. Being of differentiating nature, it could not make all the plants behave alike.

In the papers referred to I have shown that if the flora of Ceylon be divided into three groups—endemic species, those confined to Ceylon and Peninsular India, and those with wider distribution than this—the first are the rarest, the second next most rare, and the last the commonest, rare in this connexion being understood to mean occupying less area. Accepting then the view that the wides are the oldest in Ceylon,⁴ as well as the commonest, while the endemics are the youngest and rarest, the Ceylon-Indian species intermediate in both respects, I have deduced the conclusion

¹ The Endemic Flora of Ceylon. Phil. Trans., B, vol. ccvi, 1915, p. 307. The Evolution of Species in Ceylon, with reference to the dying out of Species. Ann. of Bot., vol. xxx, 1916, p. 1.

² Widely distributed in Ceylon.

³ Strictly local in Ceylon.

⁴ In the present and preceding papers I have given a good deal of evidence in favour of this view, but in a subsequent paper I propose to put it all together in one piece, as this appears to be a view which is unacceptable to many.

that area occupied goes on the average with age. I propose, in other words, to substitute *age* for natural selection as the chief agent in determining the area occupied by any given species, so long as no important barrier interferes. It must be clearly understood that in this work, as in dealing with figures of breeding under Mendel's law, one must always deal with twenty or so at once. In individual cases results may vary greatly, owing to the operation of chance, of natural selection, of local adaptation, and other factors which appear to have little or no aggregate effect in the long run or on large numbers.

The law which appears to me to govern the distribution of species may be thus tentatively expressed: 'the area occupied by any given species (taken in groups of twenty or so) at any given time in any given country in which there occur no well-marked barriers depends upon the age of that species in that country.'

Had it not been that observations on 'the rich flora of Rio de Janeiro bore out this hypothesis, and that I found that sample genera, taken at random in the floras of the Himalaya and the Malay States, conformed to the law which I have suggested, I should have hesitated to put it forward, though the evidence in its favour from the Ceylon flora (which there is no reason to consider as unique) was very strong.

The adoption of my hypothesis will be found to render simple in very many cases the puzzles of geographical distribution, such for instance as that Ceylon contains 222 widely distributed species which are each confined to a very small area, usually not over eight or ten miles in diameter, while in New Zealand (with one-fifth the flora of wides) the number is only twenty-one, several of which (see below) are fairly evident recent introductions or doubtful determinations.

By showing—as has been done for Ceylon, and will in this paper be done for New Zealand—that the distribution of plants follows simple arithmetical rules, grave doubt has been thrown upon the idea that natural selection is the main agent in determining it.¹ This position has been taken in flank, and new defences must be prepared. One of these is based upon the hackneyed argument that introduced species spread rapidly over islands at the expense of the native flora, and I have endeavoured elsewhere to show that this position is equally unsound.

This law of 'age and area', if accepted, has such important bearings upon the whole subject of geographical distribution as well as upon evolution itself, that it is highly desirable to bring up as early as possible confirmation of the results which have been obtained by a study of the Ceylon flora. A cursory investigation of many groups of plants—and

¹ i. e. so far as area occupied is concerned, which has always been supposed to be determined by natural selection. It must be clearly understood that in this work we are dealing always with area occupied, not commonness *within* the area.

animals¹—convinced me that such confirmation was easily to be obtained, and I cast about for a flora from which to procure figures as nearly like the Ceylon figures as was possible, deciding² for that of New Zealand, which for this purpose has many advantages. In the first place, it provides a test of endemism and of anything concerned therewith which by general admission has almost no rival in the world. Ceylon is not mentioned in Wallace's 'Island Life', whereas almost three chapters are devoted to New Zealand. In the second place, the flora is well known, and it is very unlikely that further exploration will seriously alter the areas in which the various species are known to occur.³ In the third place, the islands have no sudden or violent changes of climate in any part which might form a barrier to migration; and lastly, they are very convenient for determining the area occupied by any species, for they are spread out in a long curve running in general north and south for approximately 1,080 miles,⁴ with an average breadth of about 100,⁵ so that the area occupied by a species can be, and for the purposes of this work has been, roughly estimated by the distance between its extreme northern and southern limits; or rather, it would be more correct to say that longitudinal range is employed here in place of actual area. The map of New Zealand was marked by transverse lines at every twenty miles, which rendered determination simple. This method does not give close accuracy, but the islands are so narrow that the error is small, and on the total it cancels out. In a few instances the result was to place in the last class (range, 1-40 miles) species which ranged crosswise to the islands to such a distance that they should have gone into the next class above (41-160 m.). As a matter of fact, as will be seen, the figures are so conspicuously in accord with my hypothesis of 'age and area' that they would not be vitiated by a source of error considerably larger.

For the purposes of this work I have taken the flora as being that given by Cheeseman in his 'Manual of the New Zealand Flora', published at Wellington in 1906, and I have absolutely neglected all later work. In this way the facts upon which I found my conclusions are available to any one without difficulty, and, as I have explained above, detailed confirmation work does not affect the general results.

Cheeseman, however, includes in his Flora not only the plants of

¹ By the kindness of Prof. J. S. Gardiner, F.R.S., I have been able to go over certain groups of animals whose statistics of classification and distribution he considers to be reliable, and in some of these, which I have actually enumerated, I find my age and area hypothesis borne out.

² Without making any preliminary investigations whatsoever. Several botanists had said to me, 'Ceylon is all very well, but is not really a test of endemism: try New Zealand'.

³ The general effect of detailed completion work, such as most of that published about New Zealand in the last ten years (since Cheeseman's Flora), is to add new species to the lowest class of all and to raise other species, especially in the two lowest classes, in the scale, but to leave the general result unaffected.

⁴ 1,110, if the Three Kings islets at the north end be included.

⁵ In actual fact approximately 99.5 miles.

the three main islands (North, South, and Stewart, with the Three Kings islets at the north end), but also those only found in other parts of the Dominion, and even beyond, viz. in the outlying islands of the Kermadecs, separated by 420 miles to the north, the Chathams, 375 miles to the east, the Snares (60 m.), Aucklands (190 m.), and Campbells (330 m.) to the south, the Antipodes (490 m. SE.), and Macquarie (570 m. SW.). The last is officially part of Tasmania. Now these islands are as widely separated from New Zealand, and in all directions except west, as are the Maldives from Ceylon, to which they officially belong. The Maldivian flora has never been counted as part of that of Ceylon, and for scientific purposes¹ the floras of these outlying islands have no right to be counted as part of that of New Zealand, unless in thinking of New Zealand as the continental area which it once apparently was. One might with equal right include the flora of the Fatoes (180 m. away), or even Iceland (430 m.) or Norway in that of Scotland. I have therefore excluded from the flora as here dealt with all those plants² which are *only* found in the outlying islands, and have reckoned as widely distributed species, not as endemic to New Zealand, those³ which occur in them as well as in New Zealand, though nowhere else. This treatment, it is almost needless to remark, greatly reduces (from 1,000 or 1,080 to 902) the total of species counted as endemic to New Zealand, which has been unnaturally swelled by their inclusion, probably in the unconscious effort to make it seem as important as possible. If these outlying forms be reckoned as endemic to New Zealand, one should also reckon (which is now impossible) the numerous other species that analogy shows must have existed upon the land intermediate between these islands and New Zealand, which is now submerged. The tendency of workers in Europe is always to underestimate distances abroad, and I have found in a long residence in other countries that Calcutta is usually supposed to be near Colombo, Buenos Aires or the Andes near to Rio, though in each case the separation is about 1,200 miles. But to obviate any suspicion that these species have been excluded in order to bring the New Zealand flora within a certain pre-arranged scheme, it may be well to state that they bear out my age and area hypothesis in a most complete way.

In dealing with the Ceylon flora I was fortunate enough to be able to divide the wides into two groups—those found in Peninsular India (younger), and those of wider dispersal (older)—and it was the marked difference in rarity between these two groups that confirmed me in the hypothesis, till then only formed in a kind of shadowy way, that (1) age, and (2) area occupied, go together. In figures running from 1, Very Common, to 6, Very Rare, the endemics showed rarity 4.3, the Ceylon-Peninsular-

¹ For practical purposes, of course, the case is entirely different.

² In all gr (80 endemic to the islands).

³ In all 98.

Indian species 3.5, and those of wider dispersal 3.0. As these figures, or figures close to these, showed throughout the flora, family by family, and genus by genus, while at the same time every family and genus of reasonable size showed forms in every class from Common to Very Rare (i. e. occupying every size of area), in numbers increasing up or down, it seemed to me that the distribution of species in Ceylon was only explicable upon the hypothesis already mentioned. Nothing but a mechanical cause, acting equally upon all—or a combination of several causes, similarly acting without distinction upon every species or small group of species—can make all the species behave alike. Some mechanical explanation must be found, and to me the only one that seems reasonable is age.

In the case of New Zealand a similar splitting of the species may be done. East and south there lie several groups of little islands—the Chathams, Auckland, Campbells, Antipodes, and Macquarie—which are shown by the soundings to lie in such a way that the flora common to them and to Australia must, in all reasonable probability, have passed through New Zealand. In other words, the wides which reach them ought to be extremely common in New Zealand, while the endemics which occur both in them and in New Zealand must have been developed at a very early period, and ought therefore to be very common in New Zealand also (common being used as a synonym for widespread). These predictions are both borne out by the facts, and afford very strong evidence in favour of the view that the wides are the oldest, a subject to which I shall devote a later paper.

What I shall endeavour to do, then, is to show that the flora of New Zealand, like that of Ceylon, is distributed according to simple arithmetical rules; and that its plants are grouped in the same general way as those of Ceylon, showing only such differences as may be predicted from a knowledge of my age and area hypothesis. In this way I shall bring forward yet further evidence, if such be needed, against the validity of natural selection, and at the same time strengthen enormously the probabilities in favour of my hypothesis of age and area.

The flora of New Zealand, as given by Cheeseman, is thus made up :

TABLE I.

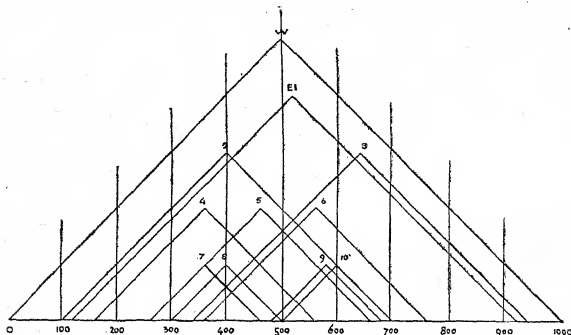
Filices (omitted in this paper)	156
Coniferae	20
Confined to the outlying islands, or to them and such countries as Australia, South America, &c. (omitted in this paper) . . .	91
Total omitted here	267
Confined to New Zealand (as defined)	902
In New Zealand and outlying islands only	98
Of wider distribution, i. e. reaching Australia, South America, &c. .	301
Total	1,568
Total here dealt with	1,301
H h 2	

As there is a widespread impression that New Zealand has a much larger endemic flora than Ceylon, and one much richer in endemic genera, it will be well to make at the start a comparison of the actual facts.

TABLE II.

	<i>Wides.</i>	<i>Endemics.</i>	<i>Endemic genera.</i>
New Zealand	399	902	23 with 31 species.
Ceylon	2,000	809	23 with 52 species.

In writing this paper I have predicted, with the aid of my hypothesis of 'age and area', what phenomena should be looked for in the flora of New Zealand, and as all my predictions have been verified when it came to examining the facts, the result has given me considerable confidence in the truth of the hypothesis. We may commence with a somewhat striking



example of this prediction and subsequent verification. As the argument is a trifle complex, I have simplified it by reducing it to the form of a diagram, which will also serve to illustrate the second prediction.

My object was to predict what (under the age and area hypothesis) would be the local distribution of the endemic species of New Zealand, assuming that the wides entered (largely, at any rate) at some part of the islands, and not indefinitely all along the chain, for which supposition there was reasonable evidence in the soundings, which show that the shallowest water approaches New Zealand towards the centre of the chain of islands.

For the sake of simplicity I have assumed that New Zealand is 1,000 instead of 1,080 miles long, and that the point of entry of the wides was at the exact centre, i.e. at 500 miles. Imagining one species (W) to enter at this point, and to follow the law exactly, its distribution may be represented by drawing a triangle showing it gradually expanding till after a certain

length of time it reaches the ends of the islands (0 and 1,000). At half time it will be at 250 and 750, and so on.

As *W* spreads, it is supposed to give rise to endemics, and the actual facts of the flora show that in most cases it does so. For this illustration I have imagined these to be formed in increasing numbers as time goes on and the *wides* occupy a larger and larger area, but there is no absolute necessity for this assumption. In actual fact also, probably some of the older—or perhaps even the younger—endemics will themselves give rise to other endemics, but as to such possibilities we have absolutely no information whatever, though now that I have shown that some of the phenomena that follow evolution can be studied arithmetically,¹ we may expect to gain a good deal of new information in such respects. I have, then, imagined all the endemics to arise directly from the *wides*, and to arise only² at the levels where that wide has reached a dispersal of 200, 400, 600, or 800 miles along the islands. I have further imagined² that at each of these levels the number of endemics will be proportional to the distribution of the *wides*, so that we shall get 1, 2, 3, and 4 arising. I then obtained the actual spots of origin by drawing numbers from a hat, when there turned up (a) 520, (b) 400, 640, (c) 560, 460, 360, and (d) 580, 400, 600, 360. Placing a dot at each of these points to represent the origin of these species, and drawing for each of them a triangle similar to that constructed for the *wides*, there appeared the result given in the diagram. The points at which the triangles meet the base line obviously represent the distances along the islands reached by these imaginary endemics when the imaginary wide has reached the ends of the islands; thus endemic No. 1 will have reached 120 and 920 at that time.

If now we divide New Zealand into ten zones, as is done in the diagram by drawing vertical lines at every hundred miles, and then count the number of triangles of endemics cut by each of these lines, we shall get the number of endemics that occur in that zone. In the particular case in hand, for instance, these numbers are

1 3 6 8 8 7 5 2 2

or if we take the number of endemics in the whole zone of 100 miles, we get

0 3 5 8 9 8 7 3 2 2

In other words, assuming that the *wides* entered more or less at some portion (not the whole) of the islands, and that the endemics were developed casually, then the number of endemics to be expected in any zone, starting

¹ By plotting series of triangles or cones like those in the diagram from the actual figures for the species of any genus or family, interesting results may be obtained, which will form the subject of later papers.

² There is no absolute necessity for either of these assumptions; they are employed simply for convenience.

at one end and going to the other, will form an ordinary rising and falling curve, rising somewhat more sharply at the beginning and falling likewise at the end, but flat in the middle. It will most often have only one maximum, but unless the endemics form rather more towards the centre than away from it, there will tend to be two maxima, with a slight depression between them. I am greatly indebted to Mr. E. G. Gallop, M.A., Mathematical Lecturer of Gonville and Caius College, for assistance in dealing with this somewhat mathematical problem.

Now to turn to the facts. Having calculated out the matter in this way, I proceeded to count up the flora of New Zealand, expecting to find that the totals at any rate would show this gradual rise to and fall from a maximum, even if it were not very regular, and perhaps showed serious breaks in continuity. In actual fact the result was very much more exact than I had ventured to expect, as will be seen at once by a glance at the following table :

TABLE III.

			Endemic Dicotyledons.	Monocotyledons.	Total.
North Island.	Zone from	0 to 100 miles	174	60	234
	"	101 to 200 "	213	67	280
	"	201 to 300 "	254	76	330
	"	301 to 400 "	280	88	368
	"	401 to 500 "	300	86	386
South Island.	"	501 to 600 "	432	105	537
	"	601 to 700 "	429	103	532
	"	701 to 800 "	424	103	527
	"	801 to 900 "	408	108	516
	"	901 to 1,000 "	322	92	414
Stewart Island.	"	1,001 to 1,080 "	98	39	137

This is a very striking table, and shows clearly that natural selection at any rate cannot be responsible for the local range. I therefore followed it up by testing the individual families and genera separately, and obtained the truly remarkable result given in the next three tables.

TABLE IV.

	North Island.					South Island.					Stewart Island.
	0-100 mi.	100-200 mi.	200-300 mi.	300-400 mi.	400-500 mi.	500-600 mi.	600-700 mi.	700-800 mi.	800-900 mi.	900-1,000 mi.	1,000-1,080 mi.
Ranunculaceae	3	7	8	13	16	21	21	28	26	15	3
Magnoliaceae	2	2	2	2	2	3	3	2	1	1	1
Cruciferae ¹	—	—	2	2	2	7	4	6	11	1	1
Violaceae	4	6	6	6	6	7	7	7	6	5	3
Pittosporaceae	11	11	11	11	8	7	6	6	5	5	1
Caryophyllaceae	—	—	—	—	1	5	7	7	7	4	—

¹ Includes a number of species confined to one, chiefly the South Island, which would, one would imagine, have spread to the North Island had they arrived at the north end of the South Island in time to get across before the separation of the two islands.

² Families showing one or more figures not in a regular progression to a single maximum.

	North Island.					South Island.					Stewart Island.
	0-100 m.	100-200 m.	200-300 m.	300-400 m.	400-500 m.	500-600 m.	600-700 m.	700-800 m.	800-900 m.	900-1,000 m.	1,000-1,080 m.
Portulacaceae	1	—	—	—	—	—	—	1	1	1	—
Malvaceae	1	1	1	1	2	3	3	3	3	5	—
Tiliaceae	4	4	5	5	6	6	5	5	5	5	3
Rutaceae	2	2	2	1	1	1	1	1	1	1	—
Meliaceae	1	1	1	1	1	1	—	—	—	—	—
Oleaceae	1	1	1	1	1	1	1	1	1	1	—
Stackhousiaceae	—	—	—	1	1	1	1	1	1	1	—
Rhamnaceae	1	1	2	1	1	1	1	1	1	1	—
Sapindaceae	1	1	1	1	1	1	1	1	—	—	—
Coriariaceae	—	1	1	1	2	2	2	2	2	2	—
Leguminosae ¹	2	3	4	5	6	14	11	14	13	7	—
Rosaceae ¹	3	4	5	5	5	8	9	8	9	7	4
Saxifragaceae	5	6	6	6	3	3	3	3	2	2	—
Crassulaceae	1	1	1	1	2	2	3	3	2	2	—
Haloragaceae	2	2	3	5	5	5	0	0	0	4	1
Myrtaceae ¹	15	15	14	14	11	12	11	5	4	4	2
Onagraceae ¹	5	5	7	6	10	13	14	17	16	14	—
Passifloraceae	1	1	1	1	1	1	—	—	—	—	—
Umbelliferae	4	9	9	11	13	24	26	26	26	19	6
Araliaceae	9	9	9	8	7	7	7	7	7	7	5
Cornaceae	4	4	4	4	4	3	3	3	3	3	1
Caprifoliaceae	4	4	2	2	2	2	1	1	—	—	—
Rubiaceae	17	17	21	22	22	25	24	21	20	17	6
Compositae	17	32	40	44	54	101	99	95	91	78	27
Stylidiaceae	—	—	2	3	4	5	5	5	6	6	3
Campanulaceae	1	1	2	2	2	3	4	4	4	3	1
Ericaceae ¹	1	1	3	4	3	2	2	3	3	3	1
Epacridaceae	5	6	8	10	11	12	15	12	12	10	5
Myrsinaceae	2	2	2	3	4	4	4	3	3	2	2
Oleaceae	3	4	3	3	3	3	—	—	—	—	—
Apocynaceae	2	2	2	2	2	2	2	2	2	2	—
Loganiaceae ¹	1	1	1	1	2	1	3	1	1	1	1
Gentianaceae ¹	—	1	1	2	3	9	9	8	9	8	3
Boraginaceae ¹	1	1	3	4	3	9	6	9	7	7	—
Convolvulaceae	1	1	1	1	1	2	1	1	1	1	—
Scrophulariaceae	6	9	16	21	24	50	55	56	52	40	6
Lentibulariaceae	3	3	4	1	1	1	1	1	—	—	—
Gesneraceae	1	1	1	1	1	—	—	—	—	—	—
Verbenaceae	2	2	2	2	1	1	1	1	1	1	—
Labiatae	—	—	—	—	—	1	—	—	—	—	—
Plantaginaceae ¹	1	2	2	2	3	2	4	4	4	3	2
Chenopodiaceae	1	1	1	2	2	3	3	4	3	2	—
Polygonaceae	1	1	1	2	2	2	2	2	2	2	—
Chloranthaceae	1	1	1	1	1	1	1	1	1	1	—
Monimiaceae	1	1	2	2	2	2	2	2	1	—	—
Lauraceae	3	3	3	3	1	1	—	—	—	—	—
Proteaceae	2	2	2	2	1	1	—	—	—	—	—
Thymelaeaceae	4	4	6	6	8	11	11	9	8	7	3
Loranthaceae	4	4	6	6	7	7	7	7	7	7	—
Santalaceae	1	1	1	1	1	1	1	1	1	—	—
Balanophoraceae	1	1	1	1	1	—	—	—	—	—	—
Euphorbiaceae	—	—	—	—	—	1	1	—	—	—	—
Urticaceae	4	4	4	4	4	2	2	2	2	1	—
Cupuliferae	1	3	4	5	6	5	5	5	5	5	—
Orchidaceae ¹	17	17	19	19	18	19	18	13	11	10	6
Iridaceae	1	1	1	1	1	1	1	1	1	1	—

¹ Families showing one or more figures not in a regular progression to a single maximum.

	North Island.					South Island.					Stewart Island.
	0-100 m.	100-300 m.	300-500 m.	500-1,000 m.	1,000-1,500 m.	0-100 m.	100-300 m.	300-500 m.	500-1,000 m.	1,000-1,500 m.	
Liliaceae	9	9	11	13	12	11	8	8	7	6	4
Juncaceae	—	1	1	2	2	4	5	5	7	3	1
Pandanaceae	1	1	1	1	1	1	1	1	1	1	—
Naiadaceae	—	—	—	—	—	—	1	1	1	—	—
Centrolepidaceae	1	—	—	1	1	2	2	3	3	3	2
Restiaceae	1	1	1	1	1	1	1	1	1	1	1
Cyperaceae ¹	22	23	24	30	26	38	34	35	36	34	13
Gramineae	8	14	18	20	24	28	33	35	40	33	12

If we take the genera with six or more endemic species, we get—

TABLE V.

	North Island.					South Island.					Stewart Island.
	0-100 m.	100-300 m.	300-500 m.	500-1,000 m.	1,000-1,500 m.	0-100 m.	100-300 m.	300-500 m.	500-1,000 m.	1,000-1,500 m.	
Clematis	3	5	5	8	7	8	7	7	5	4	1
Ranunculus	—	2	3	5	7	11	12	18	18	10	2
Lepidium	—	—	2	2	2	3	1	1	5	1	1
Pittosporum	11	11	11	11	8	7	6	6	5	5	1
Carmichaelia	1	2	3	5	6	11	8	11	11	7	—
Tillaea	1	1	—	1	2	3	3	3	3	2	1
Gunnera	1	1	2	4	4	4	5	5	5	4	1
Metrosideros	8	8	8	8	5	6	6	2	1	1	1
Epilobium	2	2	4	4	8	11	12	15	14	11	—
Azorella	—	—	—	1	2	5	5	5	5	4	1
Aciphylla	—	2	2	2	2	5	6	4	9	5	—
Ligusticum	—	1	1	1	2	7	8	9	7	6	3
Coprosma	12	12	15	16	17	18	18	16	15	12	—
Olearia	5	10	11	11	12	16	13	15	13	11	—
Celmisia	—	1	3	3	5	22	24	20	24	21	6
Gnaphalium	1	2	2	3	4	5	5	4	4	4	—
Racoula	—	2	3	3	5	10	11	11	14	8	1
Helichrysum	2	1	2	2	5	9	7	7	7	5	—
Cotula	2	3	3	4	4	7	7	10	8	9	2
Senecio	2	6	8	7	8	15	14	12	7	6	4
Dracophyllum	3	3	5	6	6	6	9	7	8	6	4
Gentiana	—	1	1	2	3	9	9	8	8	8	3
Myosotis	1	1	3	4	3	9	6	9	7	6	—
Vernonia	6	6	10	14	15	39	41	43	38	26	2
Ourisia	—	—	2	2	3	3	5	6	8	7	3
Euphrasia	—	1	2	2	3	5	6	6	5	5	1
Pinelaea	4	4	5	5	7	8	8	6	5	4	—
Fagus	1	3	4	5	6	5	5	5	5	5	—
Thelymitra	3	2	3	3	1	1	1	1	—	—	—
Pterostylis	2	3	3	4	6	7	6	3	2	2	1
Luzula	—	—	—	1	1	3	4	4	6	2	—
Gahnia	3	3	3	3	3	4	3	2	1	1	1
Carex	11	12	13	15	14	23	21	23	25	24	8
Danthonia	1	1	2	3	3	4	5	5	5	6	4
Poa	2	3	4	4	6	9	11	10	10	7	4

¹ Families showing one or more figures not in a regular progression to a single maximum.

This table was also so strikingly consistent that I followed it up by taking the genera with more than two (and less than six) endemic species.

TABLE VI.

	North Island.					South Island.					Stewart Island.	
	0-100 mi.	100-200 mi.	200-300 mi.	300-400 mi.	400-500 mi.	500-600 mi.	600-700 mi.	700-800 mi.	800-900 mi.	900-1,000 mi.	1,000-1,080 mi.	
Drimys	2	2	2	2	2	3	3	2	1	1	1	
Cardamine	—	—	—	—	—	2	1	3	3	—	—	
Meliccytus	2	3	3	3	3	3	3	3	3	2	1	
Stellaria	—	—	—	1	1	3	4	4	3	2	—	
Colobanthus	—	—	—	—	—	2	3	3	4	2	—	
Aristotelia	1	1	2	2	3	3	3	3	3	3	2	
Rubus	3	3	3	3	3	2	4	4	3	3	3	
Gemm.	—	—	—	—	—	2	2	1	2	1	1	
Acaena	—	1	2	2	2	3	3	3	4	3	2	
Myrtus	4	4	4	4	4	4	4	4	3	3	—	
Fuchsia	3	3	3	3	3	4	4	2	2	2	2	
Hydrocotyle	3	4	4	4	4	4	4	4	3	3	2	
Angelica	1	2	2	3	3	4	4	5	3	2	—	
Panax	2	3	4	4	4	4	4	4	4	4	2	
Pseudopanax	5	4	4	3	2	2	2	2	2	2	1	
Alseuosmia	4	4	2	2	2	2	2	—	—	—	—	
Nertera	3	3	3	3	3	3	2	2	2	2	2	
Lagenophora	1	2	2	2	2	2	3	3	2	2	1	
Brachycome	—	1	1	2	2	2	2	3	3	3	2	
Haastia	—	—	—	—	—	3	3	3	1	1	—	
Cassinia	2	2	3	3	2	2	2	1	1	1	1	
Abrotanella	—	—	—	—	1	2	2	2	3	2	1	
Erechtites	1	1	1	3	3	3	3	3	3	3	3	
Forsteria	—	—	1	1	2	3	3	3	3	3	1	
Gaultheria	1	1	3	4	3	2	2	2	2	2	1	
Myrsine	2	2	2	3	4	4	4	3	3	2	2	
Olea	3	4	3	3	3	3	—	—	—	—	—	
Utricularia	3	3	3	1	1	1	1	1	—	—	—	
Plantago	1	2	2	2	3	2	4	4	4	3	2	
Chenopodium	1	1	1	2	2	2	2	3	2	1	—	
Drapetes	—	—	1	1	1	3	3	3	3	3	1	
Loranthus	2	2	4	3	4	4	4	4	4	4	—	
Viscum	1	1	1	2	2	2	2	3	2	1	—	
Paratrophis	3	2	2	2	2	1	1	1	1	1	—	
Corysanthes	5	4	4	4	4	4	4	3	3	3	3	
Cordylina	3	3	4	4	4	3	3	3	2	2	1	
Astelia	4	4	4	4	3	3	1	—	—	—	—	
Schoenus	1	2	2	3	2	1	1	1	1	1	1	
Cladium	3	3	3	3	1	1	1	1	1	1	1	
Uncinia	2	2	2	3	3	5	5	5	5	5	2	
Agrostis	—	1	1	2	2	2	2	3	4	4	—	
Deyeuxia	1	2	2	2	2	2	2	2	4	2	2	
Deschampsia	—	—	—	—	1	1	1	2	2	3	—	
Triodia	—	—	—	—	—	—	1	2	3	2	—	
Agropyrum	1	1	1	1	1	1	1	1	2	—	—	

Three only of these show any break in the progression to a single maximum.

These tables are so absolutely consistent that there cannot be the slightest doubt about the matter. Nor can there be any possibility of

explaining it away, or of saying that the progression of the numbers is accidental. In only 12 families out of 70 is there *any* break in the regular progression to a single maximum, and then only in 16 figures out of 770. In any case the greatest 'error' is only 4, but in the prediction we have shown that two maxima may at times occur.

A very brief consideration of these tables is enough to show that natural selection is an agent quite incapable of causing such a simple arithmetical arrangement. The area occupied by a species cannot be due to it, though it no doubt enters to a large extent in determining the commonness of a species *within* its area of occupation.

The complete and surprising manner in which these tables bear out the prediction made (and be it noted that I made it first and verified it afterwards, thus making a discovery about the grouping of plants in New Zealand which had hitherto remained unmade) gives great support to the hypothesis of 'age and area' which I based upon the figures of the Ceylon flora.

A study of the diagram just given leads to another prediction which may be made. It will be seen that on the average the species with the longest range in the country have the lower points of their triangles towards the outer ends of the islands. Thus the only species that have one boundary in the first occupied zone, from 100 to 200 miles, have ranges in the country of 800, 600, and 400 miles (average 600), and those that have a boundary in the last zone, 900–1,000 miles, have ranges of 800 and 600 miles (average 700); whereas those with boundaries in 400–500 miles have ranges of 200 and 200, and those in 500–600 have ranges 400 and 200 (average 300). As with the preceding prediction, cases may occur in which there may be two maxima, but on an average, and on the total, one will expect to find, assuming 'age and area' to hold, that the ranges of the species will be greatest in the case of those commencing in the outermost zones, and least in the case of those commencing towards the centre. This, it need hardly be remarked, is quite inconsistent with natural selection.

Turning now to the actual facts, one finds (taking the grand total of the flora) that the range of those endemic species which have their boundaries in the different zones is as follows:

TABLE VII.

<i>Boundary (N. or S., or both).</i>	<i>Average possible range.¹</i>	<i>Actual range.</i>
Between 0 and 200 miles	980 or 100 miles.	691 miles.
" 201 " 400 "	780 " 300 "	568 "
" 401 " 600 "	580 " 500 "	321 "
" 601 " 800 "	380 " 700 "	272 "
" 801 " 1,000 "	180 " 900 "	453 "
" 1,001 " 1,080 "	40 " 1,040 "	753 "

¹ If the species ranged indifferently both ways, and not chiefly towards the centre, the average range should be the same at every point. On the theory of natural selection there is no particular reason why they should range one way more than another.

This table bears out the prediction that was made, in the most complete manner,¹ showing a decrease in the range from 691 to 272 miles, followed by an increase again to 753. A coincidence is perhaps worth noting—that the species whose range actually begins at the North Cape (0 miles) have also an average range of just 753 miles, the same as those which begin in Stewart Island.

Passing on to deal with other cases of prediction and verification, there are many other things which, if my hypothesis is correct, we shall expect to find in the numerical arrangement of the 'wides' and endemics of New Zealand. In the first place, as New Zealand is separated from the nearest land area of important size by an immense stretch of water, it is evident that few 'wides' can have arrived there in recent times (geologically speaking), though no doubt an occasional one or two have done so, and in very recent times, with the advent of the Maoris and of the white man, others have come there. The vast flora of introduced weeds may be left out of account, for there is not the least evidence to show that they would have spread had not *foreign conditions*, or disturbance of the native conditions, been *also* introduced. We shall therefore expect to find that if the flora be divided into groups, as in the case of Ceylon, according to rarity (i.e. according to area occupied), the 'wides' are, as in Ceylon, much more common than the endemics, and that both groups are arranged in graduated series, the wides being most numerous at the top, the endemics at the bottom, of the classification, since few wides are now coming in, whilst endemics probably continue to evolve.

This is exactly what we do find, as a glance at the table will show.

TABLE VIII.

Range in New Zealand, ²	Wides.	Endemics.
1. 1,001-1,080 miles	122	52
2. 881-1,000 "	79	60
3. 761-880 "	59	59
4. 641-760 "	38	61
5. 521-640 "	26	79
6. 401-520 "	27	105 ³
7. 281-400 "	18	97
8. 161-280 "	20	93
9. 41-160 "	9	128 ³
10. 1-40 "	21 ³	168 ³
	399	902
Rarity in figures from 1 to 10, corresponding to this classification . .	3-5	6-5
Equal, in figures from 1 to 6, as in Ceylon, to	2-1	3-9

(Mean rarity of whole flora by this table, 5.6.)

¹ This result also shows with individual families and genera.

² I have used ten divisions (equal, with the exception of the first and last) here to simplify calculation. Six as a number has no special virtue beyond local convenience in Ceylon, and to have used it in New Zealand would have made more difficult the classification of the species by their longitudinal range. And, further, it would be impossible to decide what divisions exactly corresponded to those used in Ceylon.

³ Explanation given below.

We may repeat the Ceylon figures for the sake of comparison.

TABLE IX.

<i>Class.</i>	<i>Wides (including those only found also in Peninsular India).</i>	<i>Endemics.</i>
VC (very common)	266	19
C (common)	580	90
RC (rather common)	416	139
RR (rather rare)	293	136
R (rare)	223	192
VR (very rare)	222	233
Rarity in figures from 1 to 6	3.1	4.3

Table VIII shows at a glance that the 'wides' and endemics are arranged as predicted, the former much the commoner, and both in graduated series, the one with numbers increasing upwards, the other with them increasing downwards.

If it be preferred to include as endemic to New Zealand those species which also occur in the outlying islands, we get the result shown in the next table, which corresponds to column H or J in the imaginary history of the Ceylon species given on p. 20 of the preceding paper in this volume.

TABLE X.

<i>Range in New Zealand.</i>	<i>Wides going beyond outlying islands.</i>	<i>Endemic to New Zealand and outlying islands.</i>
1. 1,001-1,080 miles	81	93
2. 881-1,000 "	58	81
3. 761-880 "	31	67
4. 641-760 "	31	68
5. 521-640 "	21	84
6. 401-520 "	21	111
7. 281-400 "	16	99
8. 161-280 "	17	96
9. 41-160 "	9	128
10. 1-40 "	16	173
	301	1,000

The arrangement of the plants in the different classes is very much the same as in the preceding table, but the endemics begin to show an accumulation at the top of the list,¹ though from the third class onwards they show the same graduated series as in the other table.

The regular arrangement of the endemics in the various classes from top to bottom of the scale is by itself almost enough to show that natural selection cannot be responsible for their distribution, but it may be enormously strengthened, as in the case of Ceylon, by a comparison of the different families and larger genera. These all, if of reasonable size, agree in the arrangement of their members with the total of the flora, and it will suffice to give as examples half a dozen of each, chosen by drawing the numbers from a hat.

¹ Corresponding to the greater age now dealt with.

TABLE XI.

Ranunculaceae	Wide	3	—	1	—	—	—	—	—	1
"	Endemic	1	1	3	2	3	4	6	7	7
Leguminosae	Wide	—	1	—	—	—	—	—	—	—
"	Endemic	—	—	—	—	3	3	4	4	5
Umbelliferae	Wide	4	2	2	—	2	1	—	—	—
"	Endemic	2	1	3	4	2	5	1	4	5
Liliaceae	Wide	5	—	1	—	—	—	—	—	—
"	Endemic	1	—	2	4	3	1	1	—	1
Juncaceae	Wide	4	1	4	4	—	3	—	—	1
"	Endemic	—	1	—	—	1	—	2	1	—
Cyperaceae	Wide	10	10	9	9	5	3	5	4	2
"	Endemic	10	5	2	3	4	8	8	1	8

All show the wides much commoner than the endemics, and the only one that is at all exceptional is the Cyperaceae, which shows a considerable accumulation of endemics at the top of the scale, as would be expected in the case of a family very old in New Zealand, which there is reason to believe.

The same things show in the genera.

TABLE XII.

Lignisticum	Endemic	—	—	1	—	—	3	—	3	2	6
Oleria	Endemic	—	2	3	2	3	1	4	2	6	8
Helichrysum	Wide	—	1	1	—	—	—	—	—	—	—
"	Endemic	—	1	—	—	1	3	—	3	1	5
Cotula	Wide	2	—	—	—	—	1	—	—	—	—
"	Endemic	1	1	1	1	—	—	2	1	—	5
Carex	Wide	1	5	2	3	2	1	2	1	—	1
"	Endemic	7	3	1	—	1	6	5	—	4	7
Danthonia	Wide	2	—	—	—	—	1	—	—	—	—
"	Endemic	—	1	1	—	1	—	1	1	1	4

The same thing shows in *Carex* as in Cyperaceae as a whole.

A cursory glance at these tables shows that they correspond to the Ceylon tables for families and genera given in preceding papers. All families and genera have species in all or nearly all the headings,¹ commencing from the bottom upwards, but stopping at different heights upon the scale. But all agree with the general total in their arrangement.

Opportunity may be taken here to call attention to one of the many interesting things which show in these statistical figures, and which will form the subjects of later papers. As the Leguminosae, for example, only reach up the scale as far as 640 miles (the actual range of the most extensive), it is evident that the family was on the whole late in reaching New Zealand. The only widely distributed species that it contains has a range merely of 1,000 miles (i.e. the main islands, not passing into Stewart Island). The genus *Veronica* shows the same thing, though for some reason it proved very mutable when it reached New Zealand. Or yet again, in Ranunculaceae, all the endemics in the first two classes, i.e. down to 880 miles, two out of

¹ Allowing for the small size of families and genera in New Zealand.

three in the class going down to 760, and one out of two in the class going down to 640, belong to the genus *Clematis*, which was therefore evidently an early arrival. And so on.

To proceed : as it is very probable that the land connexion to New Zealand was severed long before that to Ceylon, we shall expect to find that both wides and endemics will show comparatively few in the early or bottom stages of the table, and much greater crowding higher up, though the wides being the older will show both these phenomena more markedly than the endemics.

Both these predictions are borne out by the facts. Of the Ceylon 'wides' 222 out of 2,000, or 11 per cent., occupy areas usually not over ten miles in diameter, while in New Zealand only 21 out of 399, or 5.2 per cent., occupy areas not exceeding 40 miles in diameter. And of these 21 it is fairly certain that a part are introductions of recent date, for the number in the class above (40 to 160 m.) is only 9, or 2.2 per cent., of the wides, and the numbers, down to this class, decrease regularly. Those in this ninth class occupy areas equal to those occupied by rather common, or even common, species in Ceylon.

It is of interest to note what Cheeseman says about these 21 widely distributed species in his flora :

1. *Ranunculus parviflorus*, L., var. *australis*. 'N. Island : sheltered places on lava-streams, Mt. Wellington and Mt. Eden, &c., Auckland Isthmus ; once very plentiful but now becoming rare . . . A common Australian plant, and possibly introduced from thence in the very early days of the colony.'

2. *Hymenanthera chathamica*, T. Kirk. 'N. Island : Wellington—Patea. Chatham Islands. . . Patea specimens have neither flowers nor fruit, but appear to belong to the same species.'

3. *Aralia Lyallii*, T. Kirk. 'S. Island : Coal Island, Preservation Inlet. Stewart Island and adjacent islets. The Snares. Has precisely the habit of *Stilbocarpa polaris*, and in a flowerless state may easily be taken for it' (this species occurs on the Auckland, Campbells, Antipodes, and Macquarie). As it only occurs on one islet at the extreme south end of South Island, it may quite possibly have reached there accidentally.

4. *Senecio Stewartiae*, Armstr. (Flora, p. 378). 'Herekopere Island (in Foveaux Strait). The Snares.' Another coastal species which may have been accidentally introduced in recent times.

5. *Limosella Curdiaana*, F. Muell. (Flora, p. 489). 'S. Island : watery places in the Manuherikia valley.' This valley lies in the centre of the island.

6. *Veronica Anagallis*, L. (Flora, p. 546). 'N. Island : Hawke's Bay, watery places, Colenso. Not observed since its original discovery by Mr. Colenso more than fifty years ago. Although a widely distributed plant in the Northern hemisphere, it is unknown in the Southern except in South Africa, where it is supposed to be an introduction, and in New Zealand. Possibly Mr. Colenso's specimens were introduced as well, but if so it is remarkable that the plant should have entirely disappeared.' This was evidently an introduction.

7. *Peperomia reflexa*, A. Dietr. (Flora, p. 596). 'N. Island: woods near the East Cape.' Fairly probably an introduction.

8. *Poranthera microphylla*, Brongn. 'S. Island: Nelson—Fagus forest in the Maitai valley . . . Marlborough—Pelorus and Tinline valleys, abundant. Widely distributed in Australia and Tasmania.'

9. *Urtica australis*, Hook. f. 'No New Zealand botanist has met with it on any part of the mainland of either the North or South Island. It is not uncommon on Dog Island and other small islands in Foveaux Strait.' It occurs on the Chathams, Auckland, and Antipodes. Evidently another peculiar case.

10. *Calochilus campestris*, R. Br. (Flora, p. 686). 'North Island: Auckland—Rotorua. This doubtless has as wide a range as the following species (North and South Islands, 580 miles), but so far I have seen no specimens except from Rotorua. These exactly match the plate in Fitzgerald's "Australian Orchids", with the exception that the fimbriae on the lip never show any trace of blue, but are always red.'

11. *Chiloglottis formicifera*, Fitzger. (Flora, p. 690). 'N. Island: Auckland—Kaitia. A very remarkable little plant, previously known only from eastern Australia. . . . Specimens agree in all respects with Mr. Fitzgerald's beautiful plate.'

12. *Lemna gibba*, L. (Flora, p. 745). 'N. Island: Poverty Bay. I have seen no New Zealand specimens of this species.' Probably an accidental introduction.

13. *Lepilaena Preissii*, F. Muell. (Flora, p. 753). 'N. Island: Auckland, Waikato River, near Churchill, Kirk. I have seen no New Zealand specimen of this, but according to Mr. Kirk examples collected by him in the locality quoted above were submitted to the late Baron Mueller, and by him identified with the Australian *L. Preissii*. It greatly resembles *Zannichellia palustris* (range 660 miles, abundant in the Waikato River), and in the absence of male flowers may have been mistaken for it.' Evidently an error of determination.

14. *Lepyrodia Traversii*, F. Muell. (Flora, p. 760). 'N. Island: Auckland—swamps between Hamilton and Ohaupo, Middle Waikato district. Chatham Is., abundant in peaty swamps. A very curious species. It differs from *Lepyrodia* in the one-celled and one-seeded fruit, and was consequently erected into a separate genus (*Sporadanthus*) by F. Mueller. In its other characters and in habit, however, it is altogether a *Lepyrodia*, and it appears best to consider it a species of that genus with the ovary one-celled by abortion.' Appears a somewhat doubtful determination.

15. *Eleocharis acicularis*, R. Br. (Flora, p. 768). 'South Island: Lake Te Anau. I have seen no specimens but Mr. Petrie's, which are in young flower only. Mr. C. B. Clarke, who has examined them, states that he is satisfied that they belong to the small group consisting of *E. acicularis* and a few allied species, and most probably to *E. acicularis* itself, which is an almost cosmopolitan plant, though not yet recorded from Australia.' Evidently, it seems to me, an endemic species of the *acicularis* group.

16. *Lepidosperma filiforme*, Labill. (Flora, p. 790). 'N. Island: Auckland—clay hills between Mongonui and Kaitia. I am indebted to Mr. C. B. Clarke for identifying this with the Australian *L. filiforme* . . . will probably prove to be not uncommon north of Auckland. In Australia it has been recorded from Victoria and Tasmania.'

17. *Carex Brownii*, Tuckerm. (Flora, p. 834). 'N. Island: Auckland—marshes at Lake Tongongoe, near Kaitaia. Mr. Matthews, who is the first to observe it in New Zealand, considers it to be indigenous, and there is nothing improbable in its occurrence in the extreme north of the colony.'

18. *Imperata arundinacea*, Cyr. 'N. Island: Auckland, near Kaitaia. Perhaps introduced only, but it is one of those species which might be expected to be indigenous in the extreme north of the colony, and I have consequently given it the benefit of the doubt.'

19. *Stipa setacea*, R. Br. (Flora, p. 858). 'S. Island: Otago—Cromwell, Kurow, Dunroon, and other localities in the interior. A common Australian plant, stretching from Queensland to Tasmania. It is probably naturalized only in New Zealand.'

20. *Agrostis magellanica*, Lam. (Flora, p. 862). 'S. Island: Otago, head of Clinton valley, near Lake Te Anau. Aucklands, Campbells, Antipodes, Macquarie.' Formerly separated, but now merged in *A. magellanica* by Hooker and Hackel.

21. *Poa foliosa*, Hook. f. (Flora, p. 900). 'Confined to Stewart I.' Evidently arrived too late to cross to the South Island.

Of these 21 species, then, 5 are pretty evidently recent accidental introductions, and 5 are doubtful determinations. Two are confined to Stewart Island, and were evidently too late to reach the South Island. Two are confined to islets in Foveaux Strait, and may have accidentally reached these islets and been unable to get farther. It is also noticeable that no less than eight of the total are from Auckland Province, and of these four are from Kaitaia, and two from the Waikato. This fact makes one suspicious of introduction, as does the further fact that 12 out of the 21 are Monocotyledons. It is fairly evident that the number under this head, instead of being 21, should not be more than about 6 to 10.

In the same way, the proportion of endemics is less in the lower part of the scale than in Ceylon. Even in the lowest class, though it includes a much greater area than in Ceylon, the number is only 168 out of 902, or 18 per cent., against 233 out of 809, or 28 per cent., in Ceylon. This corresponds exactly to the fact that the wides have gone higher up the scale in New Zealand.

We shall further expect to find that the average area occupied is much greater in New Zealand than in Ceylon, for there will not be so many recent arrivals among the wides, still confined to small areas, nor so many recent formations among the endemics similarly confined.

Both these predictions are borne out by the facts. The average area occupied by a wide in Ceylon is at most about 10,000 sq. miles, while in New Zealand it is about 77,000, though New Zealand is only four times the area of Ceylon. Even the eighth and ninth classes in New Zealand occupy as much space as the average of all the wides in Ceylon.

Similarly with regard to the endemics; their rarity, in figures running

from 1 to 10, is 6.5. The sixth class of New Zealand plants contains those with a range of 401–520 miles, or double the whole area of Ceylon. The average area occupied by an endemic in Ceylon is a circle of about 40 miles in diameter, so that it is at most about one-tenth of that occupied by an endemic in New Zealand. Even the lowest class of New Zealand endemics occupies, in the case of some of its members, as much room as this.

This difference in area occupied by endemic species in the two islands is a very awkward point to explain on the theory of natural selection. New Zealand is quite as mountainous and varied as Ceylon, if not more so, yet its endemics occupy much more space. Few of them are, as in Ceylon, confined to *one* mountain (4.4 per cent. against 8.4 per cent.), though many occupy a whole range 30–40 miles long.

If, as is further probable, the land connexion to New Zealand not only ended sooner, but began sooner than that to Ceylon, we shall expect to find that both wides and endemics have on the average gone farther up the scale, and that a larger proportion have occupied the whole available area.¹ What has already been said, with a glance at the figures in the top class, is sufficient to show that this prediction also is borne out by the facts.

Turning again to Table VIII, let us examine it more carefully. It will be noticed that the endemics increase in number with remarkable regularity, except at class 6, where the number 105 is intercalated between 79 and 97, and at the last two classes, where 128 and 168 follow immediately upon 93.

The number 105 at class 6 seems to me a strong point in favour of my hypothesis, for in this class are included those endemics confined to one entire island only. Of these there are many, especially in the South Island, which has about 40 against about 6 in the North Island. The explanation appears obviously to be that most of them were held up, when they had reached the north end of the South Island, by the fact that that island was already separated from the North Island. One would therefore seem justified in subtracting from the number 105 a considerable number, perhaps 20 to 30, which would reduce it to a figure that would fit in very well with the figures for the classes above and below. It is worthy of note that of the 40 species mentioned, 10 are Veronicas, and 5 *Celmisias*.

The sharp rise in the last two classes is probably to be explained by the fact that the earlier stages of spreading take much longer than the later. If these two classes were made into three, the numbers would progress as in the rest of the series.

It is thus clearly evident that many predictions, based upon my hypothesis of 'age and area', are borne out by the actual facts in the most convincing manner. As in face of the facts that have been brought up natural selection is no longer tenable as an explanation of the geographical distribution.

¹ The same thing might, of course, happen if New Zealand was less densely covered with cryptogamic vegetation, though the land connexion was not earlier.

bution of species, so far as the area which they cover is concerned, the presumption in favour of my hypothesis is very greatly increased. There would at least seem to be fair reason to accept it provisionally, and test it, as the theory of evolution itself, and many other theories, have been tested, by applying it to the solution of other problems.

Finally, it may be pointed out that the facts of the flora of New Zealand agree so absolutely with those of that of Ceylon, that the whole argument against natural selection given in my last paper (pp. 6-16) might be repeated word for word with the necessary illustrations drawn from the New Zealand flora, as also might the argument against dying out of species other than by a geological convulsion, serious change of climate, or other important cause. There is no evidence to show that they die out under normal conditions.

Even if my hypothesis of 'age and area' be not admitted, there is no denying the fact that the species living in any particular country have been shown to follow definite numerical laws in their distribution. It therefore almost of necessity follows that that distribution—so far as area covered is concerned—is determined by causes which act mechanically upon all alike. Such causes are not numerous, and if age, which is at least an obvious one, be rejected, it will not be found easy to discover a substitute.

Geographical distribution is, it seems to me, removed from the realm of evolution proper, which is thrown back a stage. It is not a phenomenon of evolution, but a *sequel* to it. Once a species is evolved, its further history, i. e. its extension in numbers and in area occupied, appears to be, chiefly at any rate, a mechanical process, with which, so far as we can see, its past evolution has nothing to do. *How* it was evolved, or *why*, are other questions altogether. At the same time, as there seems to be an impression that this work is an attempt to destroy the theory of evolution, it will be well to point out that in reality, by showing that the species of a genus and the genera of a family are connected together by definite arithmetical relationships, it affords very great support to that theory, if support were needed for a theory established beyond cavil.

In discovering the localities given for many species, I have been very greatly assisted by H. E. the High Commissioner for New Zealand, to whom and to the staff of whose office I desire to express my most grateful thanks.

SUMMARY.

The flora of New Zealand is studied in this paper from the point of view of my hypothesis, that the geographical distribution of a species (i. e. the area which it includes within its outer localities) within a fairly uniform country not broken by serious barriers depends upon the age

of that species within that country (the species being taken in groups of twenty or so); and it affords striking confirmation of that hypothesis.

Starting from the hypothesis, numerous predictions were made as to the phenomena which should be expected to be shown by the flora of New Zealand, and as all these predictions were borne out by the facts, several new discoveries were thus made as to the geographical distribution of plants in those islands.

For example, it was predicted that if New Zealand were divided into zones of 100 miles in width, the number of endemic species would be comparatively small in the outer zones and would increase steadily towards some central point (or points). This proved to be the case, not only with the whole flora but with all the single families and genera (Tables III-VI). It was further predicted and verified that the range of an endemic species would on the average be greater the nearer that one of its limits was to either end of the islands, the facts showing (Table VII) that the range varied down from 691 miles to 272, and then up again to 753.

Other predictions made and verified were that the wides and endemics would be arranged in graduated series as in Ceylon, the former much the commoner and most numerous at the top of the scale, the latter at the bottom, not only on the total but in the families and genera (Tables VIII-XII); that both wides and endemics would show comparatively few in the early or lower stages of the scale, and greater crowding higher up; that the average area occupied in New Zealand by a species would be much greater than in Ceylon; and finally that both wides and endemics would have gone farther up the scale, and that a larger proportion would occupy all the available area.

Very strong evidence is thus adduced in favour of the hypothesis of age and area, and in any case, whether it be accepted or not, it is clear that the distribution of plants follows simple arithmetical rules, and is probably a sequel of evolution, not a phenomenon thereof.

Cytological Studies in the Protococcales.

I. Zoospore Formation in *Characium Sieboldii*, A. Br.

BY

GILBERT MORGAN SMITH.

With Plate XI and two Figures in the Text.

INTRODUCTION.

THE first account of reproduction in *Characium* is that of Braun (1) in which the process is described for several species. *Characium acuminatum* (A. Br.), De Toni, he says, has a cell structure of a pale green cytoplasm and a single pyrenoid which is located in the middle of the cell. Reproduction takes place by the gradual disappearance of the pyrenoid and a cytoplasmic division into irregular polygons. These pieces then separate, become rounded up, and elongate to form 80-150 zoospores, which are liberated by a lateral rupture of the mother-cell wall and remain in motion 2-3 hours, then affix themselves to the substratum, elongate, and become pointed. In *C. Sieboldii*, A. Br., division may take place before the cell is fully grown, the cell contents dividing transversely. There may also be a median longitudinal division of the cytoplasmic contents. Each division of the cytoplasm is preceded by a division of the pyrenoid so that every cytoplasmic fragment contains 1-4 pyrenoids. The cytoplasmic parts which have been formed by these repeated divisions are the zoospores; they vary somewhat in size and shape. The smallest number formed is 8, most frequently 16, but there may be 30-60. Microzoospores are also formed.

West (11) states that there is a repeated division of the cell contents, transverse divisions occurring before the longitudinal ones. In a short time each portion loses its angular character, becomes rounded up, and forms a biciliate zoospore. In some species the pyrenoid disappears during the formation of the zoospores and reappears upon their germination.

Lambert (5) has described the reproduction of two forms that are parasitic on Crustaceans. The chromatophore of *Characium gracilipes*, Lamb., is single, parietal, and situated in the convex side of the cell, while the pyrenoid is always a small hyaline body separated from the chlorophyll-bearing portion of the cell. The elongation of the pyrenoid is the first evidence of reproduction. It assumes a dumb-bell shape and finally separates

into two parts, each of which rounds up like the original pyrenoid. This pyrenoid division is followed by a division of the chromatophore into two parts. Repeated transverse divisions in the protoplasm produce a single row of 32 cells within the old mother-cell wall. This is followed by the longitudinal division of 30 of these cells, the two terminal cells again dividing transversely, thus forming 64 cells in all. Longitudinal division may occur, however, after 4, 8, or 16 cells have been formed by transverse division, but there is always a longitudinal cleavage of the cells at the end of the series of transverse cleavages. Lambert finds in *C. cylindricum*, Lamb., that there are two chromatophores containing oil drops, but no pyrenoids, and that the nucleus is located between the chromatophores. By repeated transverse divisions the contents of the cell form 8, 16, or 32 parts, and then there is a series of longitudinal divisions. Another type of spore-formation in which the contents of the mother-cell form 100-2,000 small zoospores is also found in this species.

MATERIAL AND METHODS.

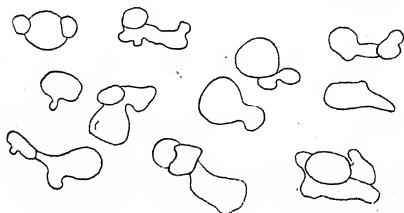
The material for this investigation was collected from a small sluggish stream on the campus of the University of Wisconsin, where the alga was found growing abundantly on blades of grass lying in the water. The shape and dimensions of the cells agree very well with *Characium Sieboldii*, A. Br. (1). The blades of grass were cut into pieces about a half-centimetre long and then dropped directly into the fixing fluid. Several different fluids were tried, but Eisen's weak solution, prepared according to the formula of Timberlake (8), proved the best. After embedding in paraffin, the material was cut on a microtome into sections from 9 to 12 microns thick. Heidenhain's iron-alum-haematoxylin gave fairly good results, but Flemming's safranin-gentian violet-orange G combination proved much better. The best preparations, however, were obtained by substituting light green FS for the orange G in the Flemming's triple stain.

THE STRUCTURE OF THE CELL.

The chlorophyll in the species studied is not located in a definite chromatophore, but apparently evenly distributed throughout the cell. The cytoplasm is not visibly alveolate in structure at any stage of development, but has always a uniform granular structure.

There is no uniformity in either the shape or location of the pyrenoid. In certain cells the pyrenoid is spherical (Pl. XI, Figs. 3 and 6), in others variously lobed (Figs. 1, 2, and 9). Although the pyrenoid of the algae is generally reported as being spherical, Timberlake (10) has found that in cells of *Hydrodictyon* that are forming starch rapidly the pyrenoids are apt to be angular, while I have found the same thing in *Scenedesmus obliquus* (Turp.), Kütz., and *S. quadricauda* (Turp.), de Breb. (7). The pyrenoid of *Characium*

always has lobed projections and never angular ones as in *Hydrodictyon* and *Scenedesmus*. At times the lobes are deeply constricted and the pyrenoid dumb-bell shaped, or there may be several lateral projections, of various sizes, that are almost completely cut off from the main body. These irregular pyrenoids are the rule rather than the exception, a few variations in shape being shown in Text-fig. 1. Usually there is one large pyrenoid near the centre of the cell, but not infrequently other pyrenoids are found scattered about in the cytoplasm. These vary in size from those that are scarcely visible to those that are 10–12 microns in diameter. The smallest ones are generally spherical and some distance from the large irregular ones, a fact which suggests their origin *de novo* rather than a formation by a division of existing pyrenoids. In the development of the cell there is a single pyrenoid at first, but later on others may appear. If there is a division of the pyrenoid it is not into two equal parts. While I am not willing absolutely to deny that new pyrenoids in *Characium Sieboldii* are



TEXT-FIG. 1. Outline drawings of pyrenoids showing their irregular contour. ($\times 2,000$)

formed by the division of an already existing pyrenoid, I am of the opinion that in a great majority of cases the pyrenoids are formed *de novo* within the cell.

Wille (12) states that the existence of starch in *Characium* is questionable. Fig. 12 shows the ring of starch plates usually found around a pyrenoid, but these differ from the starch plates in most other algae in that they are very much thinner. Owing to the comparatively large size of the pyrenoid, many starch plates are seen when it is viewed in cross-section. In certain preparations these starch plates are found just inside the plasma membrane (Fig. 12). Similar starch plates in *Hydrodictyon* have been called 'stroma' starch by Klebs (4), but Timberlake (10) has shown that these 'stroma' starch plates in *Hydrodictyon* are of pyrenoidal origin. In *Hydrodictyon* the 'stroma' starch plates are found close to the pyrenoid starch plates, but in *Characium* they are always just inside the plasma membrane. The reason for this may possibly be that the plates are so light that surface tension causes them to migrate to the periphery in much the

same manner as Czapek (2) believes that the lipoids in the cell tend to migrate towards the surface film. An indication that surface tension governs the arrangement of these small plates is seen in the transverse cleavage of the cytoplasm, when after the new plasma membrane has been formed the starch plates are located along the middle of the cell, where there were none before. If surface tension is the factor governing the distribution of these starch plates it is easily seen that with the formation of the daughter-cells there is a change in the surface tension and a readjustment of the starch plates so that they are equally distributed over the surface of the new daughter-cells. On the other hand, if these plates were dragged in mechanically by the inward growth of the cleavage furrows from the plasma membrane, there would be an unequal distribution of the starch plates and a tendency for them to be more numerous on the edges of the newly formed plasma membrane near the mother-cell wall. Since, however, the starch plates are evenly distributed along the newly formed plasma membranes the assumption that surface tension is the controlling factor in their distribution does not appear unreasonable.

The pyrenoids appear absolutely homogeneous in structure, with no light and dark areas as Timberlake (10) has shown for *Hydrodictyon* and Lutman (6) for *Closterium*. I have been able to demonstrate this irregularity in structure for *Scenedesmus* (7) and *Pediastrum*, so I believe that the failure to find the light and dark areas is not a matter of faulty technique.

Mature cells of *Characium Sieboldii* are multinucleate. The number of nuclei varies, but is always (so far as I have observed it) a multiple of two, usually 32 or 64. The nuclei are distributed throughout the cytoplasm and are approximately equidistant from each other, varying in size from $\frac{3}{4}$ to $1\frac{1}{2}\mu$ in diameter. Usually the only structures noticeable in the nucleus are the single nucleolus and a sharply defined nuclear membrane (Fig. 1), but in some cases a few chromatin granules can be distinguished (Fig. 13). These granules are comparatively large, and occasionally are connected with one another by strands. The number of granules is approximately the same as the number of chromosomes, so that it is possible that the nucleus shown in Fig. 13 is not a resting nucleus, but is in the spireme stage.

Owing to the small size of the nuclei it is impossible to make out clearly the details of karyokinesis. Nuclear division in the Chlorophyceae frequently takes place at night, but I have found no regular time for the occurrence of karyokinesis in *Characium*, and division figures are as apt to be found in material fixed during the daytime as in that fixed at night. I have not been able to determine the early stages in the formation of the spindle, but when the equatorial plate stage is reached the spindle is sharply defined. Karyokinesis takes place simultaneously in all the nuclei of a cell, this being contrary to the condition found by Timberlake (9) in the coenocytic *Hydrodictyon*, where, although all the nuclei in a cell are dividing at the

same time, those at one end are not at the same stage of division as those at the other. The mass of chromosomes in the equatorial plate is always clearly recognizable, but the spindle fibres are not seen so easily. The number of chromosomes is about 10 or 12. The individual fibres of the spindle form a cone-shaped structure that ends in a small body (Figs. 15 and 16). In some division figures the small body at either end of the spindle can be seen even though the spindle itself is indistinguishable. This small body at the spindle pole was not observed excepting during the metaphases. The nature of this body is doubtful since there are no indications of polar radiations. In the telophase figures found, the spindle could not be seen, but the two groups of chromosomes were easily recognizable, forming deeply staining masses. The details of the reconstruction of the daughter-nuclei are also lacking, but there are frequently paired nuclei in the cell, indicating cases where nuclear division has just been completed.

FORMATION OF ZOOSPORES.

There is no definite stage in the development of the alga at which zoospore development takes place. Instances were not found, however, of the formation of zoospores in cells containing less than eight nuclei. The process of zoospore formation is that which has been called 'progressive cleavage' by Harper (8). The first indication may be a single transverse cleavage of the cytoplasm, or several transverse cleavages may take place at the same time. When there is a single cleavage plane formed it is not located in the middle of the cell but at either end (Fig. 2). The process of cleavage is a furrowing in, the furrow starting at the plasma membrane and gradually growing towards the centre. Cross-sections show furrows starting towards one another from opposite sides of the cell, but the furrows probably appear at first as a ring-shaped furrow in the plasma membrane and extending entirely around the cell. In some cases the protoplasmic masses formed by the cleavage furrows contain approximately the same number of nuclei (Fig. 3), while in others the number of nuclei is decidedly unequal (Fig. 2). The position of the pyrenoids remains unchanged during the formation of the first cleavage furrows, so that whether or not a protoplasmic segment will contain a pyrenoid when it is cut off is merely a matter of chance. Fragments may also be formed in which there are two pyrenoids (Fig. 3). The number of transverse cleavage planes formed varies, but the greater the number of nuclei in the cell the greater the number of transverse planes. After several transverse cleavage planes have been formed the cleavage furrows appear in the long axis of the cell. These do not extend from one end of the mother-cell wall to the other, but are formed between two transverse cleavage planes (Figs. 3 and 4), the new furrows appearing first in the plasma membranes of the central protoplasmic masses. Furrows generally begin at opposite sides and

grow towards the centre. These furrows are broadly V-shaped and not narrow as Harper has shown them for *Synchytrium* (3). That this broadening at the outer end is the normal condition, and not due to plasmolysis, is shown by the fact that the protoplasts are not separated from one another by spaces of any considerable width along the transverse cleavage planes. At other times the secondary furrows are not formed at right angles to the primary cleavage planes but are formed at varying angles (Fig. 4). With the completion of the formation of these furrows the protoplasm is divided into a number of irregular multinucleate masses with irregular outlines. At this time also there is considerable variation in the number of nuclei in each protoplasmic mass. The fact that there is no division of the pyrenoid accompanying the cytoplasmic cleavage is much more marked after the longitudinal cleavage furrows have been formed. The pyrenoids remain unchanged during the process and are still homogeneous in structure with the ring of starch plates showing quite plainly around each (Fig. 5). The further steps in the process consist in the formation of uninucleate masses of protoplasm. The cleavage furrows that cut out these uninucleate protoplasts may be formed parallel to the long axis of the mother-cell, although they are usually formed at varying angles to the already existing cleavage planes. When the process of cleavage has been completed the whole interior of the mother-cell wall is filled with angular uninucleate masses of protoplasm (Fig. 5). These daughter-cells contain no pyrenoids, the pyrenoids of the original mother-cell remaining between the uninucleate cells in certain cases, while in others no pyrenoids can be found. The fate of the pyrenoids of the mother-cell was not determined, but their disappearance certainly occurs after the process of cleavage is well advanced.

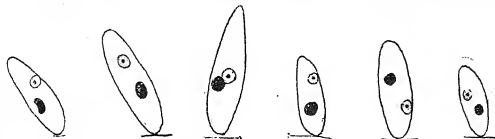
These small uninucleate cells now round up and become zoospores. Fig. 6 shows a condition in advance of that found in Fig. 5, the young cells having lost their angular shape to a certain extent, but the rounding-up process is not fully completed. This figure also shows that a pyrenoid reappears in the young cells before the rounding up has become completed. At first these new pyrenoids are very minute, red-staining bodies, when Flemming's triple stain is used, showing practically nothing of the hyaline area with which they are surrounded when the zoospore is ready for liberation. When the process of rounding up has been completed, the zoospores thus formed are ovoid or pyriform (Fig. 7), each having a pyrenoid at one end and a nucleus at the other. These two bodies are not always on the line of the long axis of the young zoospore as Fig. 17 shows. The relation of the cilia to the zoospore could not be determined from the preparations.

THE GROWTH OF THE YOUNG CELL.

Since stages in the liberation of the young zoospores were not observed the manner of their coming to rest could not be determined. From our

knowledge of other algae it would seem probable that they always come to rest with the cilia downward, and yet from the examination of the relationship between the nucleus and pyrenoid in uninucleate stages there is no definite relationship between the pyrenoid, nucleus, and base of the cell. Text-fig. 2 shows that the pyrenoid may be between the base of the cell and the nucleus, or the position of the two may be reversed, while at other times both pyrenoid and nucleus are side by side in the middle of the cell.

After the zoospore becomes attached the young cell grows considerably before becoming binucleate (Figs. 8 and 9), but after the cells have reached a certain size each is found to contain two or more nuclei, the position of which, in the binucleate cells, is quite variable, that shown in Fig. 9 being the most common. With the further growth of the cell there is a simultaneous mitotic division of the nuclei into four (Fig. 10), eight (Fig. 11)



TEXT-FIG. 2. Uninucleate cells showing that the relative position of nucleus and pyrenoid is not constant. ($\times 2,000$.)

sixteen, and so on until the maximum number of nuclei is reached. Division stages in the two- and four-nucleate cells were not found in great abundance, but in all cases found the nuclei were dividing simultaneously in each cell. In the youngest cells the pyrenoids are generally spherical, but in older cells various irregularities, that have been described above, are found. Irregularly shaped pyrenoids may be found as early as the binucleate stage, but they are not found in the majority of cells until the eight-nucleate stage has been reached. The fact that these pyrenoids are regular in the earliest stages may be considered a proof that irregularities found in the older cells are not artifacts due to the treatment during fixation and embedding.

SUMMARY.

Mature cells of *Characium Sieboldii* contain 32-64 nuclei and one or more irregularly shaped pyrenoids.

The process of zoospore formation is one of progressive cleavage, the first cleavage planes being transverse and the later ones longitudinal. The cleavage continues until angular uninucleate protoplasts are formed. The pyrenoid does not divide, but disappears during the process.

The angular protoplasts become zoospores by rounding up, forming pyrenoids and cilia.

After coming to rest the zoospore develops without cell division into the new plant, but the nuclei increase in number by simultaneous division as the cell enlarges.

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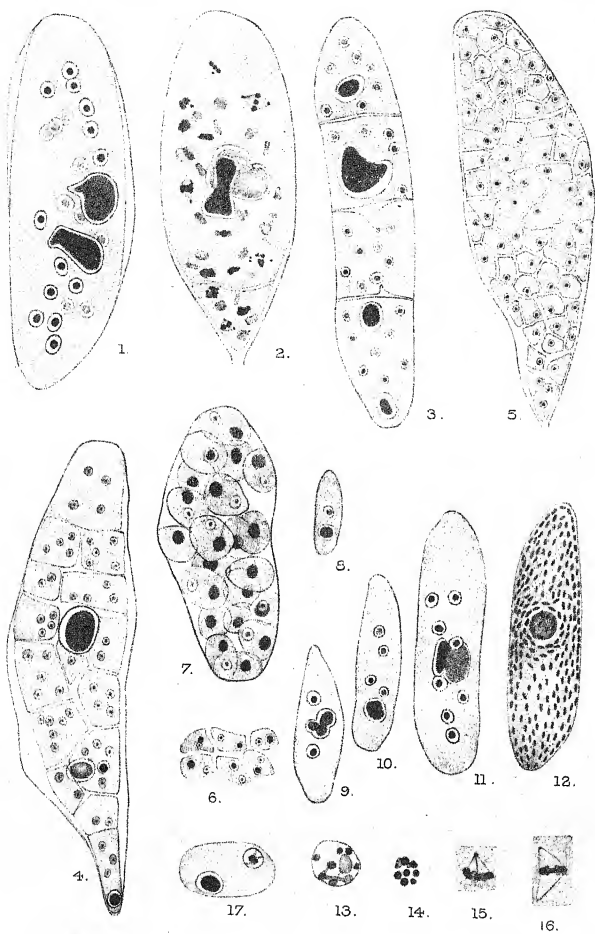
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DESCRIPTION OF FIGURES ON PLATE XI.

Illustrating Mr. G. Morgan Smith's paper on *Zoospore Formation in Characium Sieboldii*.

All the figures were drawn with the aid of the Abbé camera lucida, the drawings being made at the level of the base of the microscope. Figs. 1 and 7 were drawn with the Leitz oil-immersion objective $\frac{1}{8}$ and ocular 4 (magnification about 2,000 \times); while in Figs. 2-6 and 8-12 the ocular 3 was used (magnification about 1,650 \times). Figs. 13-17 were drawn with the Zeiss 2 mm. objective, 1.40 N.A., and compens. oc. 18 (magnification about 3,800 \times).

- Fig. 1. Mature cell showing position of pyrenoids and nuclei.
- Fig. 2. Cell with simultaneous division of nuclei and the beginning of cleavage.
- Fig. 3. A stage at the beginning of longitudinal cleavage.
- Fig. 4. Showing the cleavage into several multinucleate masses.
- Fig. 5. After the cleavage into uninucleate masses.
- Fig. 6. Uninucleate masses partially rounded up to form zoospores.
- Fig. 7. Young zoospores nearly ready for liberation.
- Figs. 8-11. Stages in the development of the young colony.
- Fig. 12. A cell showing 'stroma' starch.
- Figs. 13-16. Stages in nuclear division.
- Fig. 17. Nearly mature zoospore.



O. M. Smith, del.

Huth, London.

SMITH — CHARACIUM SIEBOLDII, A.Br.

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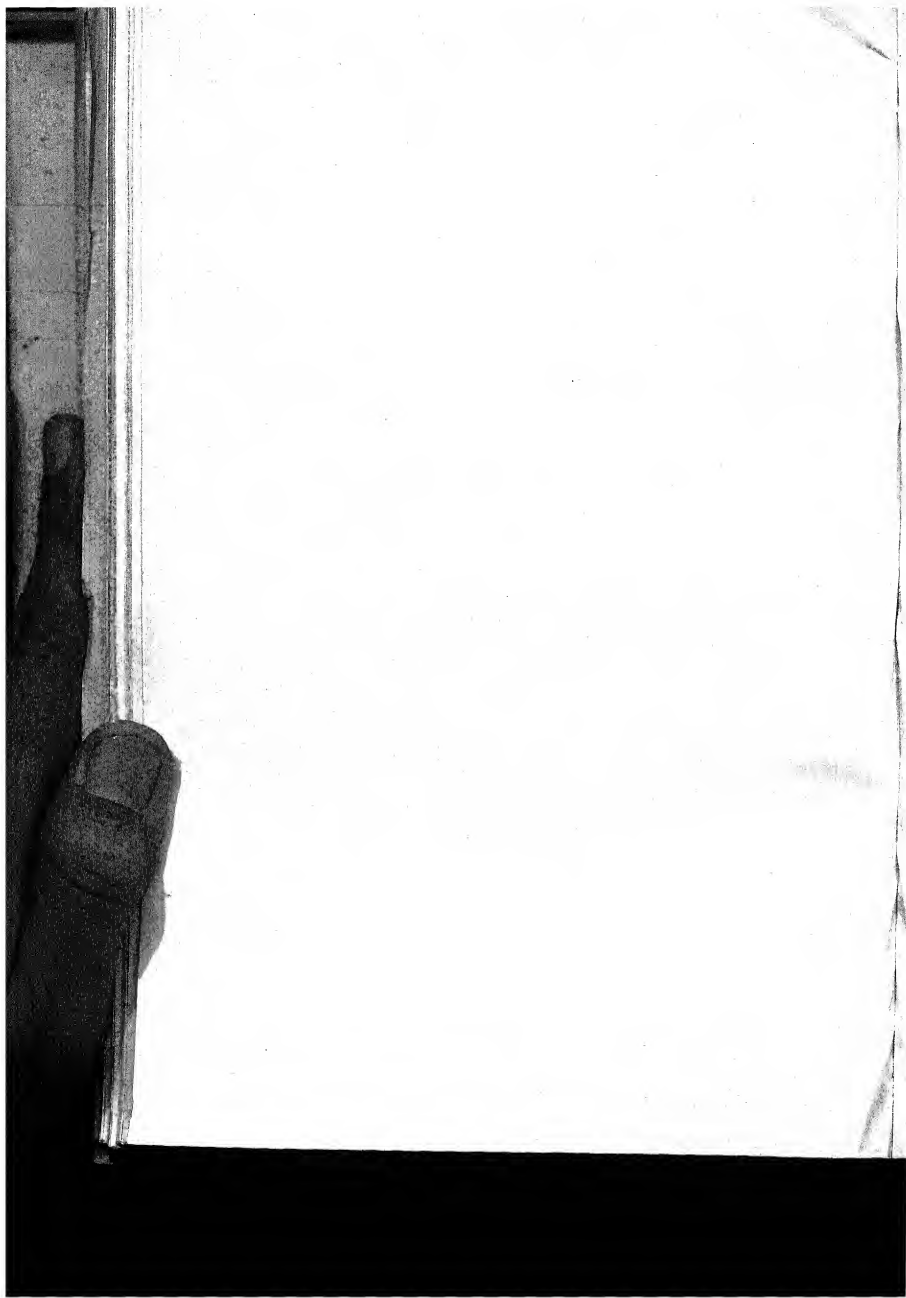
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Cytological Studies in the Protococcales.

II. Cell Structure and Zoospore Formation in *Pediastrum Boryanum* (Turp.), Menegh.

BY

GILBERT MORGAN SMITH.

With Plate XII and four Figures in the Text.

THE family Hydrodictyaceae of the Protococcales is of exceptional interest because of the peculiar formation of daughter colonies by means of motile zoospores. The cytological details of the process have been studied in *Hydrodictyon* by Timberlake (17), Klebs (7), and others; in *Euastropsis* by Lagerheim (8); but nothing is known regarding the process in *Pediastrum*. This is due to the fact that although the alga is widely distributed in nature, it rarely occurs in sufficient abundance to fix for cytological study. Even general laboratory cultures do not produce it in abundance, so that only by the application of special culture methods is a sufficient number of colonies obtained to embed in paraffin.

The source of the alga, whose study is reported in the present paper, was the plankton of Lake Mendota at Madison, Wisconsin; and it was obtained in pure culture by means of the methods which I have described in another connexion (14). The cultures were grown in 0.2 per cent. Knop's solution, and fixed in Flemming's weak osmic-acetic-chromic acid mixture diluted with an equal volume of water. The washing and dehydration of the material were by means of osmosis through a celloidin film placed over the end of a vial in the manner which I have described elsewhere (12). The material was embedded in paraffin, and cut on a microtome in sections 5-8 μ in thickness. Flemming's triple stain gave the best differentiation of the pyrenoid and a good differentiation of the nucleus. Heidenhain's iron-alum-haematoxylin also stains the nucleus satisfactorily.

Practically all descriptions of colony formation are based upon the observations of Braun (2). He found in *P. Boryanum* that the division of the mother-cell contents and the liberation of the young colony took place in the afternoon. According to his account, the cell divides into halves by cleavage. The halves then redive, but not always simultaneously.

Intermediate stages between the primary and the completed cleavage to form sixteen zoospores are not described. After the zoospores have been formed they are discharged through a longitudinal split in the mother-cell wall, the innermost layer of which forms a vesicle into which the zoospores are extruded. Immediately after their extrusion the zoospores begin to move rapidly inside this vesicle. At first they form an irregularly shaped mass, but after about fifteen minutes they come to rest forming a flat plate of cells. When the movement of the zoospores ceases they are slightly emarginate. An hour after, the peripheral cells are still further emarginate, and after four hours the emarginations have developed into horns. During the first few hours in the life of the colony there are spaces between the individual cells, but at the end of twenty-four hours the cells are closely applied to one another, and the horns have the same shape as in mature cells. The vesicle surrounding the young colony persists for a short time only.

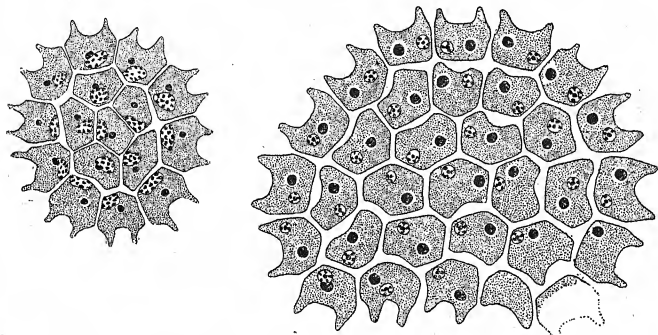
Askenasy (1) is the only one who has studied the cell contents in preparations of *Pediastrum*. He finds that in preparations of *P. Boryanum*, cells 9–13 μ in diameter, there is a single eccentrically located nucleus some 2 μ in diameter, while cells 13–18 μ in diameter are either uni- or binucleate. With the growth of the cell there comes an increase in the number of nuclei, so that a large number is present in mature cells. He was unable to study nuclear behaviour during the cleavage stages of zoospore formation because the entire contents of the cell took the haematoxylin stain so deeply, but he assumes that each zoospore contains a single nucleus. Askenasy states that the pyrenoid can be recognized in very young cells, and that as the cell increases in size there is a corresponding increase in the size of the pyrenoid. Surrounding the pyrenoid is the usual ring of starch plates; there is also starch in the cytoplasm. He was unable to follow the history of the pyrenoid after the first cleavages in zoospore formation, but found that the first cleavage plane either passed through the pyrenoid, dividing it into two unequal parts, or left it undisturbed.

In the youngest cells that I have observed there is always a single nucleus and pyrenoid. The nucleus is, as Askenasy (1) has shown, markedly eccentric (Pl. XII, Fig. 1), but the pyrenoid has no definite position. In spite of its very eccentric position within the cell, the nucleus has no definite relation to the margin of the colony. It may be on the side of the cell towards the periphery of the colony, on the side towards the centre, or it may be lateral (Text-fig. 1). With further cell growth the position of the nucleus becomes more central, until at the maximal size for uninucleate cells it is frequently central.

The structure of the nucleus, in spite of its minuteness, is much more like that of the nuclei of higher plants than I have found to be the case in other Protococcales (12, 13, 15). There is always a single nucleolus and

a sharply defined nuclear membrane. The chromatin and the linin content vary, however, at different stages in the life history. In very young cells the chromatin granules are pronounced and near the nuclear membrane, but the linin threads can be distinguished only with difficulty (Pl. XII, Fig. 1). At this time the nucleolus is less conspicuous than in the later stages. As the cell becomes older the nucleolus and the linin threads grow more prominent, whereas the chromatin granules become less conspicuous, but never so indistinct as not to be recognizable in good preparations (Fig. 21, 22).

Although many of the details are lacking, there is sufficient evidence to show that the nuclear divisions are mitotic. In the equatorial plate stage a distinctly bipolar spindle can be recognized. The chromosomes are arranged



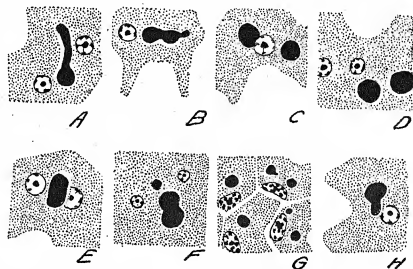
TEXT-FIG. 1. Young colonies showing that the nucleus and pyrenoid of a cell are not definitely located with respect to the margin of the colony. ($\times 3,000$.)

in a dense group when observed from the side (Fig. 2), but in polar views they appear much more distinctly (Figs. 4, 5). Although Timberlake (17) describes a small, spherical, densely staining body at each pole of the spindle in the closely related *Hydrodictyon*, I have not observed anything of the sort in *Pediastrum*. Since no cells were found in which some of the nuclei were dividing and some were not, it may be safely assumed that the nuclei within a cell divide simultaneously.

The cytoplasm of the youngest cells is granular, but a little later scattered vacuoles appear (Fig. 3). These vacuoles never become a conspicuous feature, however, and do not fuse to form a large central vacuole. As the cell becomes still older they frequently disappear, so that the mature cell again seems to be filled with a dense granular cytoplasm (Figs. 5, 6). The cell grows to a considerable size before the first nuclear

division takes place (Fig. 2). After the cell becomes binucleate there is still further growth before the next divisions occur (Fig. 4). Askenasy (1) states that the mature cells of *Pediastrum* contain large numbers of nuclei, but my observations show that the number is rarely greater than four, although 8- and 16-nucleate cells have been found. The nuclei in the mature cell lie equidistant from one another in the central portion of the cell. Since all the cells of a colony are of the same age, it might be expected that karyokinesis would take place in them simultaneously; I have never found this to be the case, however, since it often happens that some of the cells in a mature colony are binucleate and others tetranucleate.

Pyrenoids are present in the youngest cells. Usually but a single one appears, although cells with two, and even three pyrenoids, have been observed. There is no relationship between the size of the cell and the



TEXT-FIG. 2. Portions of cells showing irregularly shaped pyrenoids. G shows that very young cells may contain two pyrenoids.

number of pyrenoids, since the occurrence of more than one pyrenoid is as frequent in young as in old cells (Text-fig. 2). The shape of the pyrenoid varies considerably. Spherical ones are most abundant, angular ones are by no means rare, and in isolated instances they have small bud-like extrusions or projections. Timberlake (16) has regarded angular pyrenoids as evidence of rapid starch formation in *Hydrodictyon*, but in the case of *Pediastrum* the angular and the spherical pyrenoids are found in cells of the same colony. Pyrenoids with bud-like projections (Text-fig. 2) are of interest as being possible stages in the division of the pyrenoid; but I am of the opinion that such is not the case, and that this peculiarity is merely an irregularity in the shape similar to that which I have found in *Characium* (15). When stained with Flemming's triple stain the pyrenoid is normally a homogeneous, red-staining body surrounded by angular starch plates which stain blue (Fig. 10). I have never found the pyrenoid staining blue, as McAllister (9) finds to be the case in *Tetraspora*. Cases have been found in which one

portion of the pyrenoid stained and the other did not (Figs. 3, 6). These may possibly represent stages in a metamorphosis of the body of the pyrenoid into starch similar to that described by Timberlake (16); but since the unstained portions are always a part of the pyrenoid and never a segment that has been cut off, I am inclined to consider them as due to irregularities in staining and not as stages in starch formation. The starch plates which surround the pyrenoid are curved, and usually three or four are visible when it is viewed in median optical section. The space always perceptible between the starch plates and the pyrenoid appears empty. This space is not always of uniform width, as I have found in *Characium* (15) and *Scenedesmus* (13), but may be much wider at one side than another (Figs. 5, 10, 22). There is also considerable stroma starch scattered throughout the peripheral region of the cell, especially in colonies from older cultures in which it occurs in abundance. In preparations destained sufficiently to show the nuclei well, the 'stroma' starch plates are frequently so decolorised that the cytoplasm appears to consist of fine angular vacuoles (Fig. 4); but proper staining demonstrates that what seem to be vacuoles are really 'stroma' starch plates (Fig. 7). This accumulation of starch plates in the cytoplasm is not peculiar to *Pediastrum*, but is a constant characteristic of old cultures in all the Protococcales that I have had under cultivation. The accumulation of starch is accompanied by a loss of the chlorophyll in the plant, so that the general appearance of the culture is not a bright fresh green but a sickly yellow-green. The same loss of colour and accumulation of starch may be seen in the cells of *Oedogonium*, *Spirogyra*, and other filamentous forms collected in the field during autumn. Cells containing these excessive amounts of 'stroma' starch are not normal, as is shown by the fact that such cells in the filamentous species fail to divide, and similarly in cultures of Protococcales, where the cells contain large amounts of starch, this same pathological condition is evidenced by the small number of young, recently formed colonies.

Askenasy (1) calls attention to the fact that there is a definite relationship between the size of the pyrenoid and the size of the cell, since increase in size of the cell is accompanied by an increase in size of the pyrenoid. I have observed this relationship in both the cells with the single pyrenoids and in those with more than one pyrenoid. In the latter there is the growth of the pyrenoids as the cell grows, but the rate of growth is much slower than in cells with but a single pyrenoid.

The colonies of *Pediastrum* are not invested with a gelatinous sheath as are those of many Protococcales. This fact can be demonstrated by using Schröder's (11) method of staining living material with vesuvin, or by Errera's Indian ink method (4). Various systematic works figure and describe the walls between adjacent cells as being red, while the outside

walls of the marginal cells are colourless. I have observed this red coloration of the walls of the interior cells in living material when using the lower magnifications (500–750 diameters), but have never found it with the higher ones (1,500–2,000 diameters); I consider this red coloration therefore an optical illusion.

In preparations stained with Flemming's triple stain the walls appear to be composed of two parts: a very thin outer layer which takes the gentian violet and a thick orange-staining portion (Fig. 8). The portion stained by the gentian violet is not always of uniform thickness, but is likely to be much wider at the angles of the cells. The inner orange-staining layer does not show unless the preparations are very heavily counterstained with the orange G, so that in most preparations this inner part does not stain, but appears as a clear space between the violet-stained portion of the wall and the cytoplasm (Figs. 4–6). At first glance, therefore, many cells appear to be slightly plasmolysed when they are really turgid.

Petersen (10) has recently found in various species of *Pediastrum*, including *P. Boryanum*, that there are long tufts of bristles protruding from each cell in the colony. In a few instances he was able to see these bristles in living material, but by means of special staining methods he demonstrates the presence of these bristles in several different species where they cannot be found by examining the living material. I have tried Petersen's staining methods on the colonies of my cultures with negative results. His material, however, came directly from the plankton, and it is quite possible that these bristles are not developed except under plankton conditions.

In my cultures I have observed a certain diurnal periodicity in swarm-spore liberation. The alga was observed for the entire twenty-four hours of the day, and with very rare exceptions swarming occurred only at daybreak. Swarming colonies first appear about an hour before sunrise, but the maximum amount takes place just at sunrise. These observations are not in accordance with those of Braun (2), who found swarming only in the late afternoon. The precise hour of swarming varies from month to month, the maximum occurring at 5.30 a.m. in May and at 7.30 a.m. in December. That the induction of swarming is very largely dependent upon daylight can be demonstrated readily by placing a portion of a culture in a dark room before the first signs of daybreak (2–3 a.m.) and leaving the remainder of the culture where it will be illuminated by the rising sun. At the hour of sunrise the illuminated culture shows active swarming, while the unilluminated one shows none; but if the latter culture be brought out into the light after the illuminated culture has ceased swarming, it too will show a large number of swarming colonies. This relationship between the swarming period and the illumination was first observed by Braun (2) in *Hydrodictyon*.

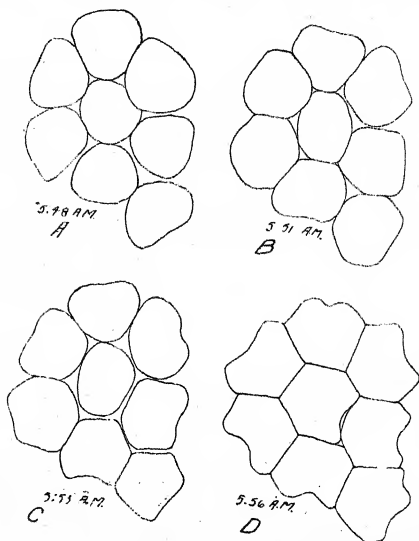
The formation of the swarm-spores and the number of cells in a colony are greatly influenced by cultural conditions. Sixty-four- and thirty-two-celled colonies predominate among those produced during the first two weeks in cultures containing 0.2 per cent. Knop's solution, while in cultures a month old 16- and 8-celled colonies predominate. At the end of two months few new colonies are formed, the cells of the old colonies in the culture containing large amounts of 'stroma' starch. The production of colonies with large numbers of cells in fresh vigorous cultures, and the later production of those with fewer cells, is a fact that has been noted by all investigators of coenobitic algae under cultural conditions.

The observations of Caspary (3) and Braun (2) have shown that any cell in the colony is capable of giving rise to a daughter colony. Since cells in the same colony are of exactly the same age and have been under the same external environmental conditions, the natural expectation would be that all cells would form zoospores at the same time. Any one familiar with field collections of *Pediastrum* knows, however, that generally at most only a few cells of a colony are empty as a result of zoospore formation. The question arises, then, whether the other cells of the colony will form zoospores on some succeeding night, or whether, on the other hand, only a few cells of a colony will ever produce them? Upon observation, however, it was found that zoospores are formed and liberated from different cells in the same colony at different times (Fig. 17).

Since my observations agree in the main with Braun's (2) account of the swarming, little need be said on this point. In my cultures, however, I find that the changes taking place after the swarm-spores come to rest are much more rapid than Braun describes them. The rapidity with which these changes take place is shown in Text-fig. 3, A-D, which are outline camera lucida drawings made at three-minute intervals. These sketches should be regarded as being only approximate, since in each instance less than a minute was devoted to the outlining of the eight cells in the colony. The sketches show that within the few minutes following the cessation of zoospore motion changes take place in the cell that according to Braun's description require a period of hours for their completion.

For the observation of the cytological details of zoospore formation I selected month-old cultures which were forming 8- and 16-celled colonies, because the small number of nuclei made the process comparatively easy to follow. The details are essentially the same as in the formation of 32- and 64-celled colonies. The first stages in zoospore formation are found in the early hours of the night, and the first indication of what is to occur is a rapid division and rediision of the nuclei so that their number is increased fourfold. These nuclear divisions are always simultaneous; the number of nuclei present is therefore always a multiple of two. Nuclear division takes place only in the cells that are to form zoospores, so that a surface view of

a colony shows 16-nucleate cells adjacent to 2- and 4-nucleate ones (Text-fig. 4). There are two general types of nuclear behaviour previous to spore formation in Algae and Fungi: either a succession of nuclear divisions of a single large nucleus in a mature cell, or nuclear division as the cell grows, resulting in a multinucleate condition when mature. In the latter case there is no nuclear division just previous to spore formation. The former method is followed by *Synchytrium* and *Ulothrix*; *Rhizopus*, *Characium*, and



TEXT-FIG. 3. Outline drawings, at three-minute intervals, of the changes taking place in the first few minutes after the cessation of zoospore movement. In this colony the swarming lasted eight minutes, and the first drawing was made fifteen seconds after the motion ceased. ($\times 2,000$.)

Hydrodictyon follow the latter. In *Pediastrum* there is a combination of the two types, inasmuch as the cell is multinucleate, but just before zoospore formation a period of active nuclear division sets in, the resulting nuclei lying a short distance from one another within the cytoplasm. At times there is a grouping of the nuclei into fours (Figs. 11, 19), but at other times this grouping is not apparent (Fig. 9). The grouping of the nuclei in fours and eights has been observed in *Hydrodictyon* by Timberlake (17), so that

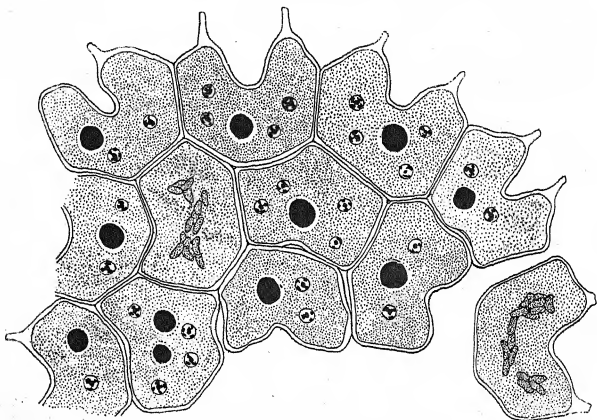
there may possibly be a specially active period of nuclear division just prior to zoospore in *Hydrodictyon*. The appearance of the nuclei of *Pediastrum* at this time is quite different from the appearance at other periods. Before this period of nuclear division sets in there is a conspicuous nucleolus, the chromatic material being quite faint and lying near the nuclear membrane (Figs. 19, 22); but after the rapid nuclear divisions the peripheral chromatin granules are very conspicuous (Figs. 9, 13).

While these nuclear changes have been going on the pyrenoid has disappeared. In the various algae whose spore formation has been studied the behaviour of the pyrenoid is not uniform. In zoospore formation in *Hydrodictyon* (7, 17) and *Cladophora*, and in spermatogenesis in *Sphaeroplea* (5, 6), the pyrenoids generally disappear before spore formation sets in. On the other hand, in the zoospore formation in *Characium* (15) and *Tetraspora* (9), and colony formation in *Scenedesmus* (13) and *Tetradismus* (12), in which the products of cell-division are the morphological equivalents of zoospores, the pyrenoid does not disappear, but remains unchanged in one of the daughter-cells. I find in *Pediastrum* that it is only in exceptional cases (Fig. 12) that the pyrenoid persists after the process of cleavage commences. Stages were found in the disappearance of the pyrenoid, and it was noted that the ring of starch plates disappears while the body of the pyrenoid becomes diminished in size, frequently becoming quite angular just before its final disappearance (Figs. 11, 12). Cases were not found similar to those described by Askenasy (1), in which the first cleavage plane cuts through the pyrenoid, dividing it into either equal or unequal parts.

The first cleavage plane may be so placed as to include either the long or the short axis of the cell. Whether it is formed by a furrowing in of the plasma membrane or by the formation of a row of vacuoles could not be satisfactorily determined, but I am inclined to think it takes place by the former method. In a very few instances cleavage planes that did not extend clear across the cell were found between two neighbouring nuclei (Fig. 13). These suggest conditions similar to those found in *Hydrodictyon* by Timberlake (17), in which cleavage furrows appear at various places between the nuclei. Unlike the furrows in *Hydrodictyon* those in *Pediastrum* do not branch. The protoplasts formed by the cleavage furrows are always angular, and the number of nuclei in each varies from one to four (Figs. 14, 15, 17). The process of cleavage continues until the entire contents of the cell have been cut up into angular uninucleate pieces (Fig. 17). Mention has been made of exceptional cases in which the pyrenoid persists after the beginning of cleavage. In two instances, when the triple stain was used, a small red-staining body was found in one of the uninucleate protoplasts (Fig. 16), which may possibly be the last vestige of the pyrenoid. The nuclei in the cells that are undergoing cleavage have much the same

appearance as those in which it is about to occur, the densely-staining chromatin being a conspicuous feature of all such nuclei.

The formation of uninucleate protoplasts is generally completed by midnight. At this time each nucleus lies at one of the angular apices of its protoplast. The nuclei are frequently lenticular or curved instead of spherical at this time (Fig. 17). The next step in the formation of the zoospores is a rounding up and swelling of the protoplasts so that the cleavage planes entirely disappear. When this rounding up begins the nuclei are even closer to the edge than they were when the protoplasts were still angular (Fig. 16). As the cleavage lines disappear the nuclei



TEXT-FIG. 4. Surface view of a colony showing 16-nucleate cells adjacent to 2- and 4-nucleate ones. The cleavage planes have disappeared in the two cells that are forming zoospores. ($\times 2,000$.)

become more dense, but the chromatin granules and the nucleolus are still distinguishable (Figs. 18, 19).

In material fixed at 2 a.m. no trace of cleavage planes could be found, but many cells similar to those shown in Figs. 18 and 19 were found in my preparations. Cells in the same colony stain differently, so that all gradations can be found between those in which there is a heavy stain in the cytoplasm and those in which the cytoplasm is only faintly stained; yet in no instance do any cells in this material show signs of cleavage planes. The objection might be raised that this condition which I have interpreted as a swelling of the protoplasts, making the cleavage planes indistinct, comes really before and not after cleavage. However, cells

without cleavage planes are never found in preparations having angular protoplasts, but are always found in material fixed at a time after the cleavage into uninucleate protoplasts has been completed. Even stronger evidence upon this question is afforded by the condition of the cell contents, in that the nuclei are dense and irregular after the cleavage furrows have disappeared, and pyrenoids are never found in these cells (Fig. 19 and Text-fig. 4). On the other hand, cells are sometimes found in which there has been the preparatory division of the nuclei, but in which for some reason there has been no cleavage. These cells are easily distinguished from the stage just described by the less dense, spherical nuclei and the presence of a pyrenoid (Fig. 22, a).

The next noticeable change is the reappearance of the cleavage planes. These look in section like very fine lines, and at the time that they reappear the protoplasts are fully rounded up. In a few instances the planes made their appearance at one end only of the cell (Fig. 21), but in the majority of cases the process of reappearance seems to be simultaneous in all parts of the cell (Fig. 22, b). At this time the nuclei are even more elongate and dense than at any previous stage in the cleavage process, and the chromatin granules can only be distinguished with the greatest difficulty. These rounded, uninucleate protoplasts are the zoospores.

After the reappearance of the cleavage planes the protoplasts separate slightly from one another, the nuclei remaining flattened (Fig. 20). I am in doubt concerning the very last developmental stages, since in the fixed preparations it is impossible to say whether a definite cavity contains zoospores or zoospores that have germinated into cells. In certain instances there is no doubt that the zoospores have germinated into cells within the mother-cell wall (Fig. 24). On account of these difficulties it is impossible to determine whether the nuclei, which are very much flattened when the cleavage planes reappear, become rounded up in the zoospore stage or after the zoospores have germinated to form cells. Nor am I certain at what precise step the pyrenoids, which are always found in the youngest cells, reappear.

SUMMARY.

The youngest cells of *Pediastrum Boryanum* are uninucleate, each containing one, rarely two or three, pyrenoids. Mature cells contain four or eight nuclei and one to three pyrenoids. The nuclei increase in number by simultaneous division so that the number is always a multiple of two. In structure the resting nucleus differs but little from the nucleus of the higher plants.

The pyrenoids are homogeneous in structure and surrounded by curved starch plates.

Previous to zoospore formation there is a period of active simultaneous nuclear division resulting in 16, 32, 64, or 128 nuclei within the cell.

The zoospores are formed by cleavage. The cleavage is progressive, forming first multinucleate protoplasts and later uninucleate ones.

The pyrenoid disappears previous to or during the first stages of cleavage.

After cleavage is completed the nuclei become dense, and the line of demarcation between the protoplasts disappears, reappearing shortly before the zoospores are liberated.

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EXPLANATION OF FIGURES ON PLATE XII.

Illustrating Mr. G. Morgan Smith's paper on *Cell Structure and Zoospore Formation in Pediatrum Boryanum*.

All the figures were drawn with the aid of the Abbé camera lucida, the drawings being made at the level of the base of the microscope, and with the Leitz oil-immersion objective $\frac{1}{4}$ and ocular 4. The magnification is about 2,100 diameters.

- Fig. 1. A young cell showing position and nature of nucleus.
- Fig. 2. Metaphase in the first nuclear division.
- Fig. 3. Young binucleate cells.
- Fig. 4. The second nuclear division. The vacuolate appearance of the cytoplasm is due to unstained 'stroma' starch plates.
- Fig. 5. Metaphases in the third series of nuclear divisions.
- Fig. 6. Eight-nucleate cells.
- Fig. 7. A cell showing 'stroma' starch plates scattered throughout the cytoplasm.
- Fig. 8. The appearance of the wall when deeply stained.
- Fig. 9. A sixteen-nucleate cell.
- Fig. 10. Pyrenoids of different forms and their surrounding starch plates.
- Fig. 11. Showing the disappearance of the pyrenoid and the aggregation of the nuclei in fours.
- Fig. 12. Showing the beginning of cleavage before the disappearance of the pyrenoid.
- Figs. 13-15. Multinucleate protoplasts formed by first cleavage furrows.
- Figs. 16-17. The completion of the cleavage into uninucleate protoplasts.
- Figs. 18-19. Showing the disappearance of the cleavage lines and an apparent return to the pre-cleavage condition.
- Figs. 20-23. The reappearance of the cleavage lines.
- Fig. 24. Colony formed by the germination of the zoospores without liberation from mother-cell wall.
- Figs. 25, 26. Ripe zoospores or very young stages in germination of zoospores within mother-cell wall.

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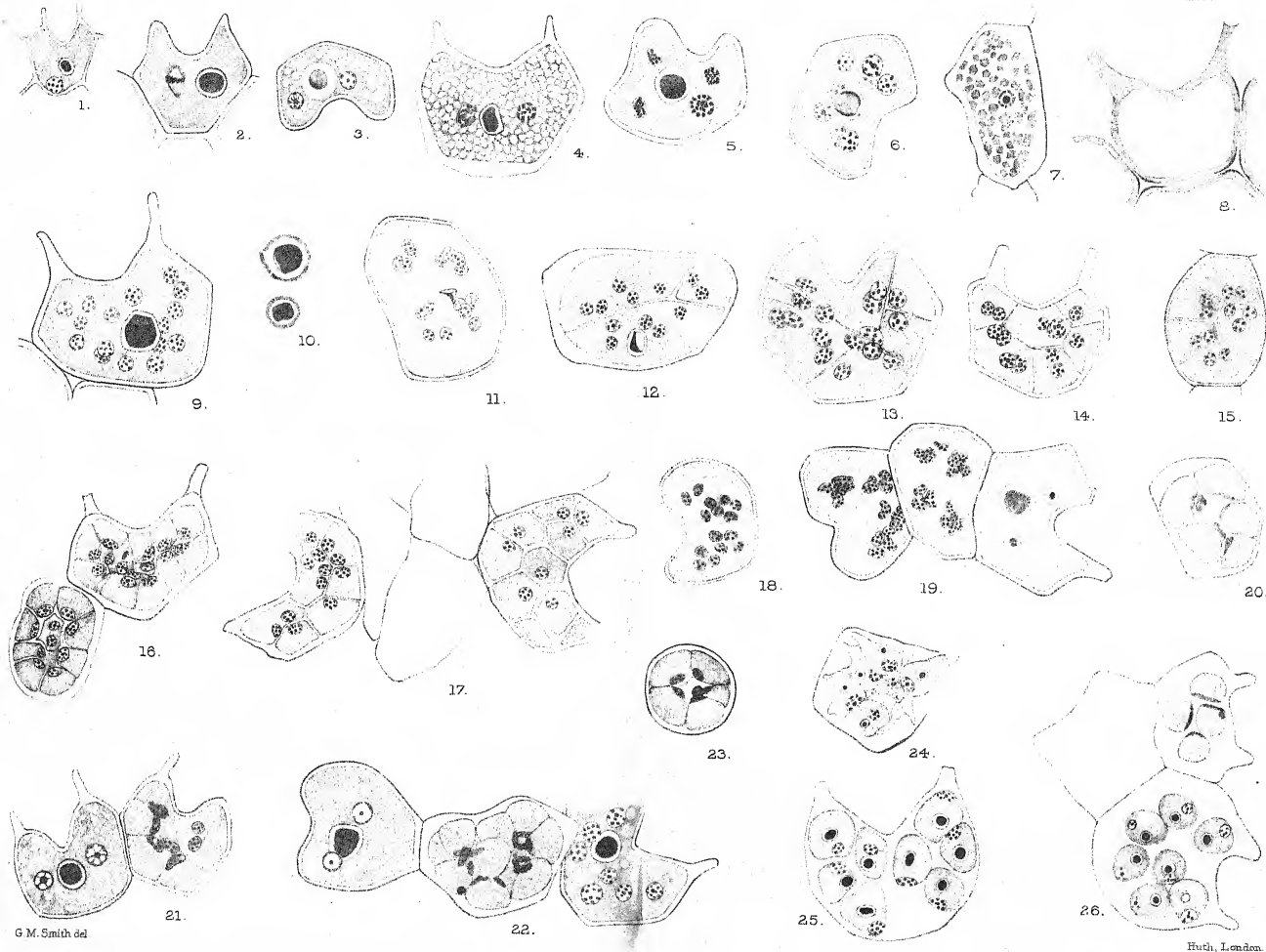
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SMITH — PEDIASTRUM BORYANUM.



NOTE.

A NOTE ON AN ABNORMALITY IN THE STEM OF *HELIANTHUS ANNUUS*.—The stem of the Sunflower has been the subject of such frequent detailed examination that it is surprising that so few cases of abnormal structure have been recorded. This is also the case with many other plants which have been selected as types for laboratory work. In the course of such treatment large quantities of material are cut up and examined, with the result that every opportunity is provided for gaining a knowledge of the range of variety of structure. The uniformity of the results of such examinations serves only to emphasize the remarkable constancy with which the typical structure is maintained.

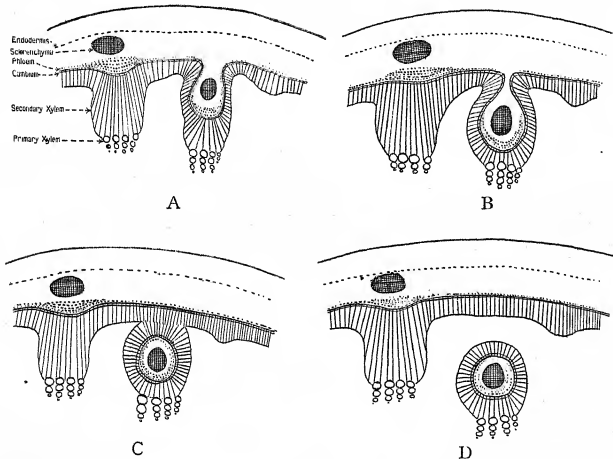


FIG. 1.

The particular variation in structure met with in this instance was found in a short length of stem taken from a young plant of *Helianthus annuus*.

Unfortunately only a small portion of the material showing the abnormality was found, and it did not include a node. However, there were sufficient differences in structure between the two ends of the specimen to suggest a possible explanation of the mode of origin of the peculiarities about to be described.

[Annals of Botany, Vol. XXX. No. CXIX. July, 1916.]

Two distinct abnormalities were presented by the specimen :

1. A deviation from the normal longitudinal course of one of the leaf-trace bundles.

2. A development of secondary vascular tissues in connexion with the displaced bundle resulting in the formation of a concentric bundle.

1. A reference to the diagrams (Fig. 1) illustrating the series of sections taken through the length of the material under examination will show that one of the

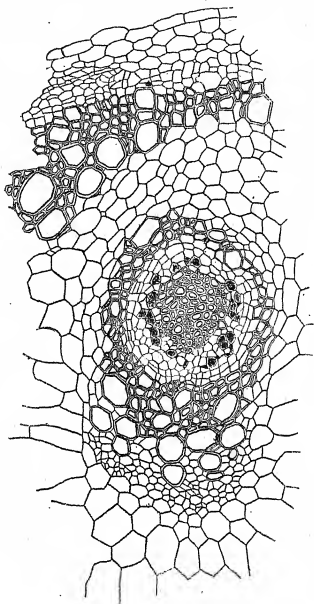


FIG. 2.

bundles of the normal vascular ring, presumably a leaf-trace bundle, departs somewhat from the typical course.

Instead of taking a vertical direction parallel to the neighbouring bundles, it passes obliquely downwards and inwards until it is in the pith, completely separated from and interior to the other vascular strands.

How far this abnormal course is continued it is impossible to say, owing to the

lack of material, but it may be presumed that the bundle eventually returns to the periphery and establishes connexions with the neighbouring bundles at some node below.

The course of this bundle suggests, to some extent, that taken by the leaf-traces in some members of the Piperaceae, where, it will be recalled, the leaf-trace bundles form a system of medullary bundles during a part of their course in the stem.

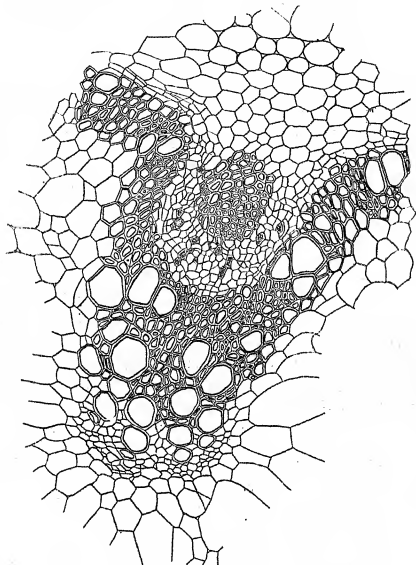


FIG. 3.

It is not suggested that the anomalous bundle in *Helianthus* traverses a complete internode before passing into the pith, as is the case for example in *Peperomia*.

2. The development of secondary tissue in the vascular ring in the neighbourhood of the abnormal bundle is of considerable interest.

A relatively small number of secondary elements have been laid down, and consequently there has been very little displacement of the primary tissues (Figs. 2 and 3). A reference to the diagrams in Fig. 1 will make clear the sequence of events.

At the stage illustrated in Figs. 1, A, and 3, the bundle is not far removed from the

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normal ring, and the interfascicular cambium has developed between the bundles, and has consequently taken a somewhat sinuous course in this region.

In successive sections this sinuous course becomes more pronounced, the interfascicular cambium taking a radial course and passing along the flanks of the sclerenchyma (Fig. 1, B). The further removal of the bundle from the normal ring leads to closer approximation of the horns of the U-shaped band of cambium, until they are separated by no more than two or three intervening cells.

Apparently the activity of the cambium at these points has been limited to the development of xylem. Quite suddenly the cambium is short-circuited both above the sclerenchyma and at the level of the normal ring, the middle portion and the intervening cells differentiating at once into xylem elements (Fig. 1, C). As a result of this, a meristematic zone is completed around the pericyclic sclerenchyma of the displaced bundle, and at lower levels the activity of this cambium has resulted in the formation of concentric layers of both phloem and xylem elements, the latter being continuous with the xylem of the normal ring.

Figs. 1, D, and 2 represent the bundle in its most internal position, and an examination of Fig. 2 shows clearly that here the bundle has been too far removed from the normal ring to exert any influence on the development of the ordinary interfascicular cambium. The latter has bridged the gap between the neighbouring bundles directly, and a normal development of secondary tissues has resulted. It is clear, however, that the activity of the cambium within the bundle has not remained uninfluenced by the stimulus of the dividing cells which are giving rise to the interfascicular cambium, since a formation of meristematic cells along the flanks of the sclerenchyma has resulted here also in the establishment of a closed ring of cambium.

This cambium has been responsible for the laying down of both secondary phloem and xylem in such a way as to result in the production of an amphivasal concentric bundle, quite isolated from the normal vascular ring.

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PREFATORY NOTE TO TWO UNPUBLISHED PAPERS
BY THE LATE PROFESSOR D. T. GWYNNE-VAUGHAN.

AMONG the research notes of Professor Gwynne-Vaughan on the vascular system of the Pteridophyta two investigations were in a sufficiently forward state to justify publication.

In both cases the idea of the research was clearly defined, full notes of the observations were available, and the slides were annotated with a view to the preparation of figures. We have thus found it possible to present the results almost entirely in the author's own words, and to add illustrations in accordance with the original intention.

The paper on 'The Anatomy of the Leaf in the Osmundaceae' existed as a preliminary draft, and it is published substantially without alteration, but with the addition of photographic illustrations. This is the paper referred to in Part V of the series of memoirs on the fossil Osmundaceae.

In the case of the investigation on 'Some Climbing Davallias and the Petiole of *Lygodium*' only the introduction had been drafted. This contains, however, a clear statement of the object of the work, and the facts extracted from the full notes on the several species become in its light a connected story. It is from this point of view that we have included some already known facts as to *Lygodium* which were reinvestigated by the author for purposes of comparison with *Davallia*. The notes include a series of freehand pencil sketches, photographs of some of which, after being traced with Indian ink and bleached, are here reproduced as text-figures. We have supplemented them by photographs of a few of the more important sections.

Had these papers been completed by Professor Gwynne-Vaughan the theoretical bearings of the facts would doubtless have been more fully developed and placed in relation to the work of other investigators. We have preferred to make no attempt to go beyond what was actually set down except in respect of the few notes enclosed in square brackets and the description of the figures in the text and plates. The course we have adopted avoids the disadvantage of any attempt to rewrite the papers.

Though unfinished in expression, each of them represents in all essentials a completed investigation. Their relation to the author's previous work and to the developments proceeding from it will be evident to students of plant anatomy.

Professor Gwynne-Vaughan's anatomical preparations, including all those on which his published work was based, have found their appropriate home in the Botanical Department of the University of Glasgow. The numbers cited below in relation to the figures and photographs refer to the catalogue of this collection.

H. C. I. G.-V.

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Observations on the Anatomy of the Leaf in the Osmundaceae.

BY

D. T. GWYNNE-VAUGHAN.

With Plate XIII

IN a paper published in 1908¹ by Dr. R. Kidston and the author, on the origin of the adaxially curved leaf-trace of the Filicales, it was suggested that the changes in form exhibited by the leaf-trace of the Osmundaceous fossil *Thamnopteris Schlechtendahlia*² as it passes through the cortex of the stem indicated the actual manner of the evolution of the typical filicinean C-shaped trace. According to this view it is assumed that all the various forms of the leaf-trace in the Filicales may be traced back to a single ancestral type already possessing a dorsiventral symmetry. This primitive leaf-trace contained a solid central mass of xylem, more or less elliptic in transverse section, the short axis of the ellipse corresponding to the median plane of symmetry of the leaf. The protoxylems were immersed (mesarch), and, at a still earlier stage, it may be assumed that they were derived from a single median strand. Phloem probably occurred on both sides of the xylem.

From this simple leaf-trace it is possible to derive all the various forms found in the Zygopterid petioles and also the adaxially curved, C-shaped leaf-trace in all its protean disguises. The Zygopterid line of evolution starts by the substitution of parenchyma for the *lateral tracheae on the outside* of the two protoxylems, so as to give rise to two lateral islands or bays.³ The C-shaped line of descent is initiated by replacing the *tracheae on the adaxial side* of the protoxylems by parenchyma, so as to give rise to a single adaxial island or bay.

[Figures illustrating this hypothesis are given in the paper on the Origin of the Adaxially Curved Leaf-trace¹ and in the Fossil Osmundaceae, Part IV.³]

¹ Gwynne-Vaughan and Kidston: On the Origin of the Adaxially Curved Leaf-trace in the Filicinae. Proc. Roy. Soc. Edin., vol. xxviii, Part vi, 1908, p. 433.

² Kidston and Gwynne-Vaughan: On the Fossil Osmundaceae, Part III. Trans. Roy. Soc. Edin., vol. xlv, Part iii, 1909, p. 651, Pl. IV.

³ Kidston and Gwynne-Vaughan: On the Fossil Osmundaceae, Part IV. Trans. Roy. Soc. Edin., vol. xlvii, Part iii, 1910, p. 469.

The ancestral fern leaf is regarded as having a single row of branches on each side of the rachis. It follows, therefore, that the double row of branches on each side of the petiole possessed by some *Zygopterideae* is held to be a special development evolved within this affinity and one probably related to the assumption of an erect habit of growth by the leaf and the consequent arrangement of the lateral appendages in a more or less radial manner to avoid overshadowing.

We may now consider the way in which the lateral branches of this primitive leaf may be assumed to have received their vascular supply from the rachis. It seems probable that, when about to give rise to a branch-trace, one of the lateral protoxylems would elongate in the direction of the long axis of the ellipse and then divide. A small mass of xylem would then protrude from the lateral margin of the trace, enclosing the outer protoxylem, and this would eventually be constricted off as the branch-trace. In the *Zygopterid* affinity this conjecture receives a considerable amount of support, because in the petiole of *Asterochlaena*,¹ which is on all grounds still fairly close to the common ancestor, a process very near to this is known to take place, the only complication being that the lateral islands of parenchyma are already present.

It was to see whether the C-shaped leaf-trace still retained any features which might indicate the primitive method of branching that the following investigation was made.

The manner in which the vascular supply of a branch is given off from its mother-axis in the *Osmundaceous* leaf varies in the same petiole according to the order and position of the branch in question. The simplest methods are to be found where the smaller veins depart from the midrib of the lamina or where small secondary pinnae arise on the rachis of a primary branch. In these cases the xylem strand of the trace is too small and thin to exhibit the features of special interest present in the branching of the stouter but still quite small traces to which our attention will be mainly directed. In the very simple branchings referred to above, the xylem strand of the trace may be reduced to an almost straight transverse band, as in *Osmunda Claytoniana* (Pl. XIII, Photo 1), or it may still form a well-curved crescent, as in *Todea barbara*. In either case the endarch protoxylem on the side of the branching divides into two (Photo 1) and the xylem strand constricts between the two protoxylems so as to nip off the outer of them for the trace of the branch (Photo 2).

In somewhat larger though still quite small branchings, when the outline of the trace varies from elliptic to more or less reniform in transverse section and the xylem strand is a fairly stout and well-curved C with two to four groups of protoxylem, the procedure is most often as follows. As the

¹ Paul Bertrand: Structure des stipes d'*Asterochlaena laxa*, Stenzel. Mém. de la Société Géologique du Nord, t. vii, p. 1. Lille, 1911.

point of branching is approached from below, the end of the xylem strand of the trace on the side of the branch becomes somewhat thickened. The protoxylem strand on that side also elongates laterally and prolongs itself into the thickened extremity of the xylem strand so that it becomes immersed. There is now a certain amount of the metaxylem of the thickened end of the strand on the adaxial side of the protoxylem; this adaxial xylem may be regarded as centripetal (Photos 3 and 5). The elongated protoxylem now divides into an inner and an outer lateral protoxylem, the two becoming separated by a few elements of metaxylem (Photos 3 and 6). The inner protoxylem is still endarch, touching on the concave margin of the xylem strand, but the outer is mesarch, being completely immersed in the metaxylem of the thickened and laterally prolonged end of the same. The outer protoxylem, together with the metaxylem lying to the outside of it laterally, is now nipped off by the incursion of a groove or furrow of parenchyma beginning on the adaxial side and gradually passing through the thickened end of the xylem strand of the parent trace to the abaxial side (Photos 4 and 7).

Sometimes this groove of parenchyma begins so soon that it reaches the immersed protoxylems before they have become separated from each other by metaxylem. The inner protoxylem is in this case for a while in contact with parenchyma both on the inside and the outside, and there is an isolated mass of centripetal metaxylem on its adaxial side (Photos 8 and 9). This soon joins up with the abaxial metaxylem on the outside of the inner protoxylem, which thus becomes normally endarch again.

In some cases of branching the lateral protoxylem of the xylem strand passes completely into the thickened end and becomes definitely mesarch (Photo 10). In these cases it may divide as before, the outer protoxylem passing off with the branch-trace, the inner opening out into the concavity of the parent trace and becoming endarch once more. The inner protoxylem may, however, still remain mesarch for some distance after the departure of the branch-trace before becoming endarch (Photo 11).

In some cases the mesarch protoxylem does not divide at all, but passes out as such with the trace. Here, of course, the xylem strand of the mother axis will have one protoxylem less above the branch than it has below (Photos 12 and 13).

In the smaller branchings of this type, when the end of the xylem strand is but slightly thickened, the centripetal elements on the adaxial side of the dividing protoxylem are very few—three, two, or only one (Photo 14). It is obvious that these are transitional stages to the simplest type of branching already described.

Another type of branching, which must be regarded as an advance on the type just described, is usually found in somewhat larger branchings. When the lateral protoxylem of the mother axis is prolonged laterally into

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the thickened end of the xylem strand it is accompanied by the soft tissues that line the concavity of the trace. Sometimes these are represented by a few layers of the xylem sheath lying on the adaxial side of the protoxylem and forming a bay between it and the centripetal metaxylem. In more advanced cases the phloem and pericycle also behave in the same way. Accordingly just below the departure of a trace the xylem strand is more or less strongly bulged outwards on that side (Photos 15 and 17). The protoxylem divides as before, and at about the same time the curve of the xylem breaks across on its adaxial side a short distance from its extremity (Photos 16 and 18). The small mass of xylem thus left behind gradually becomes joined on to the rest of the xylem strand, usually a little to the outside of the inner protoxylem, so that the latter continues to be endarch. More rarely the adaxial xylem joins on immediately in front of the inner protoxylem, so that this becomes temporarily mesarch.

This adaxial mass of xylem can only be regarded as representing the centripetal xylem in the previously described method of branching. It is interesting to note that in some cases it also may become very scanty, being reduced to one or two tracheae only (Photo 19), thus furnishing another method of transition to the simplest type of branching first described.

The further development of the method of branching is related in quite a simple manner to the increase in size of the parent trace and of the trace of the branch. As the latter becomes larger the bay becomes wider and its central region becomes invaded by the endodermis and the central ground tissue of the parent trace, so that the departure of the branch-trace leaves a gap in the parent trace communicating directly with the external ground tissue. This is the case in *Todea barbara*, *Todea superba*, *Osmunda bipinnata*, and *Osmunda javanica*. If the branch-trace is curved into a ring at the point of attachment, as in *Osmunda regalis*, it opens out a short distance above. In *Todea hymenophylloides*, *Osmunda regalis* var. *palustris*, and *Osmunda regalis* var. *japonica* the parent trace is not interrupted even at its largest branchings.

The position in the leaf at which any particular type of pinna-trace departure is to be found varies greatly from one plant to another and even from leaf to leaf, and seems to depend a good deal upon the stature and vigour of the leaf.

The simplified or reduced type of branching can arise on the one hand by the loss of the centripetal xylem on the adaxial side of a mesarch protoxylem immersed in a solid mass of xylem, or on the other hand by loss of the xylem mass on the adaxial side of a bay of parenchyma. Since the method of branching with a bay of parenchyma is derived from that with a solid thick-ended xylem strand the two methods are connected and the missing adaxial xylem is the same in the two cases.

In considering the relative primitiveness in the structure of the trace in the different regions of the leaf a great deal of caution must be exercised. It seems clear that the highest complexity in structure is to be expected, and indeed is actually to be found at the base of the free petiole, for this region must negotiate the water-supply for the whole leaf and must also bear the strain of the total weight unassisted by the stem. The leaf-trace in the cortex of the stem is relieved of the duties of mechanical support and is free to retain more primitive characters. In the upper region of the rachis, at any rate above the first lateral branches, and in these branches themselves the duties both of support and of conduction progressively diminish, and here again a simpler construction would at any rate be feasible.

So far as the structure of the trace itself is concerned this certainly holds good in the Osmundaceae. Starting from a point just above its base the petiole may be said to indicate broadly its phylogeny in two directions, both upwards and downwards.

With regard to the manner of branching, however, other factors come into play and the matter is on a different footing. Speaking generally the method of branching becomes more primitive on passing higher up the main rachis and in its lateral branches. Still it must be remembered that in some species the lowest branches of the rachis and of the primary branches are themselves reduced in size, being markedly smaller than some of those higher up. This reduction affects the method of branching so that it may present features of a more or less primitive type.

The apical pinnae of the rachis and its branches are about the same size and have about the same work to perform as the earlier leaves of the young sporeling. In consequence, as we proceed *down* from the apex of the leaf we may expect to meet very much the same series of changes in the vascular system as we should if we were examining the young leaves of the sporeling passing from leaf to leaf *up* the stem. So far as we have been able to follow it this is indeed the case in *Osmunda*, with one distinction, that the thickening of the xylem at a branching and the immersion of the protoxylem are omitted in the sporeling. It may be that the phylogeny of the earlier leaves is distinct from that of the later leaves and that, owing to the small size of their traces, these features could not appear.

In the largest branchings of *Osmunda regalis* the median axis of symmetry of the branch-trace is almost at right angles to that of the parent trace. It is rather less inclined in the other species of *Osmunda*. In all the smaller branchings it becomes still less inclined and may become quite parallel to the axis of symmetry of the parent trace. In *Todea* the median axis of the branch-trace is actually at right angles to the parent trace in large branchings and is still inclined to it at a wide angle even in the smaller branchings, though it may become almost parallel in *Todea hymenophylloides*.

The facts described in this paper have an evident bearing on the regions of the C-shaped trace. If the above interpretation of the phenomena be accepted it is clear that in the xylem strand of the petiolar trace in the neighbourhood of the smaller branchings we have to deal with three distinct regions:

- (a) the abaxial curve of the xylem strand;
- (b) a lateral portion which is going to pass out into the branch;
- (c) a mass of centripetal xylem that is going to remain in the mother trace.

This makes a line drawn across the adaxial points of departure of the branch-traces a very important distinction,¹ because it divides the parent trace into portions corresponding to the abaxial (centrifugal) and adaxial (centripetal) halves of the presumed ancestral trace. In the larger trace these regions are still present, all enlarged but unequally so. The greatest extension is experienced by the abaxial curve, but the centripetal xylem also increases in volume and may acquire a protoxylem or even two of its own.

DESCRIPTION OF THE FIGURES IN PLATE XIII.

Illustrating Professor Gwynne-Vaughan's paper on *The Anatomy of the Leaf in the Osmundaceae*.

(All these figures are from untouched photographs.)

px. i., inner protoxylem remaining in the parent trace; *px. o.*, outer protoxylem departing with the branch-trace; *px.*, undivided protoxylem; *ad. xy.*, the adaxial or centripetal metaxylem.

Photos 1 and 2. *Osmunda Claytoniana*. Origin of trace to small secondary pinna. No adaxial xylem present, the branch-trace being simply nipped off. ($\times 140$.) Slide No. 1982.

Photos 3 and 4. *Todea superba*. Origin of trace to ninth pinna from the base of the leaf. ($\times 140$.) Slide No. 2022.

Photos 5-7. *Osmunda Claytoniana*. Three stages in the division of the protoxylem and origin of the pinna trace to a fairly large secondary pinna. ($\times 140$.) Slide No. 1978.

Photo 8. *Todea barbara*. Preparation for origin of the trace to the seventh lowest pinna on a fertile branch of the leaf. The separating groove of parenchyma reaches to the undivided protoxylem. ($\times 140$.) Slide No. 1994.

Photo 9. *Todea hymenophyllodes*. More advanced stage of a similar origin of a pinna trace. The xylem of the branch-trace is about to separate. ($\times 140$.) Slide No. 2025.

Photos 10, 11. *Osmunda regalis*, var. *palestis*, horticultural variety *congesta*. The origin of the traces to the top free pinnae of two branches. In Photo 10 the protoxylem has become definitely mesarch and is dividing. In Photo 11 the branch-trace has passed off, but the inner protoxylem remains mesarch. ($\times 140$.) Slide No. 1948.

¹ It corresponds, I believe, with a line passing through the 'marges' of C. E. Bertrand.

Photos 12 and 13. *Todea barbara*. Origin of trace to the ninth lowest pinna (sterile) on a fertile branch of a leaf. The protoxylem does not divide, but passes off with the branch-trace. ($\times 140$.) Slide No. 1996.

Photo 14. *Todea superba*. Origin of trace to the pinna of a branch of a leaf; the adaxial metaxylem is reduced to a single trachea. ($\times 140$.) Slide No. 2015.

Photos 15 and 16. *Todea hymenophylloides*. Origin of trace to twenty-second main pinna from top of a leaf; some of the soft tissues lining the concavity of the xylem of the main trace accompany the protoxylem when preparing to divide. ($\times 140$.) Slide No. 2024.

Photos 17 and 18. *Todea superba*. Origin of trace to the second smallest top branch of a leaf. Similar to Photos 15 and 16, but the bay filled with soft tissues is more marked. ($\times 67$.) Slide No. 2012.

Photo 19. *Osmunda bipinnata*. Origin of trace to the third undivided pinna at the top of a leaf. There is a bay of soft tissues, but the adaxial xylem is reduced to two tracheae. ($\times 140$.) Slide No. 1971.

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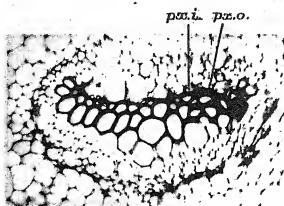
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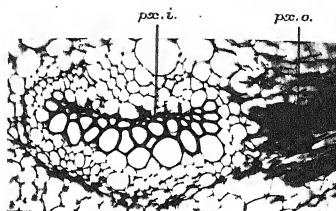
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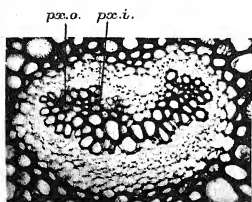
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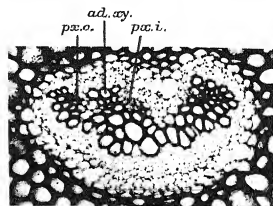
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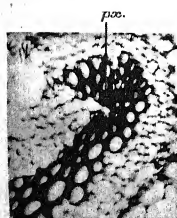
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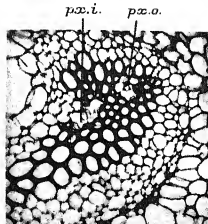
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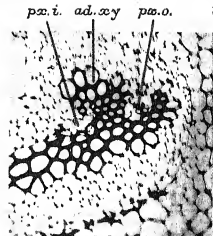
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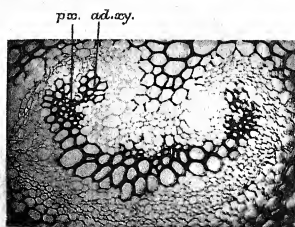
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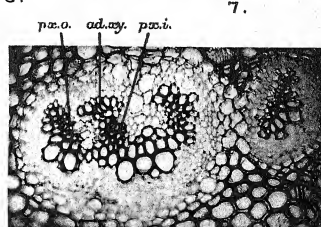
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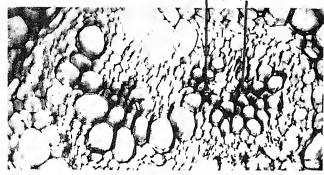


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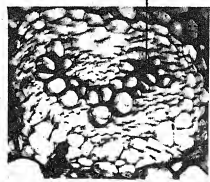
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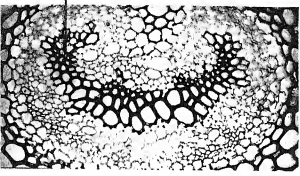
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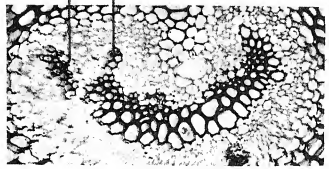
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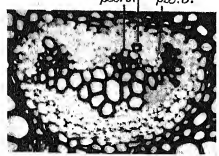
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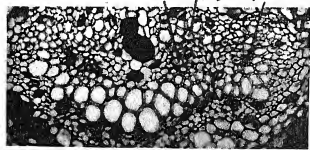
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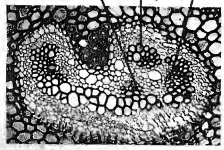
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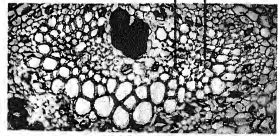
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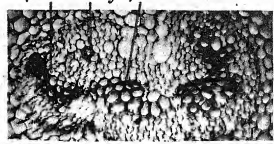
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On some Climbing Davallias¹ and the Petiole of *Lygodium*.

BY

D. T. GWYNNE-VAUGHAN.

With Plate XIV and eight Figures in the Text.

RECENT studies in the structure of the earlier fossil Filicales have lent considerable support to the theory that all the different forms of petiolar trace in the group are to be derived from a single primitive and ancestral type. This hypothetical trace is imagined as more or less round or elliptic in section, with a solid central mass of xylem of the same outline, and possessing originally a single mesarch protoxylem.

It has been shown how both the Osmundaceous² and the Zygopterid³ type of trace can be derived from this primitive form, and recently M. Paul Bertrand has shown⁴ that the Botryopterid type may also be derived from it, and, directly or indirectly, the Anachoropterid type.

As regards the living orders of Ferns, it seems probable that their petiolar traces are all variants of the Osmundaceous type with the form of an adaxially curved C. The traces of the Polypodiaceae, Cyathaceae, and Gleicheniaceae conform fairly readily with this type, and, in spite of certain difficulties, so it is believed will those of the Hymenophyllaceae and Marattiaceae. In the Schizaeaceae *Aneimia* provides us with examples of very typical C-shaped traces, but, according to the text-books, those of *Lygodium* and *Schiaaea* are of quite a different type, and in no way related to the C.

¹ [The only species described in the notes is *D. fumaroides*, material of which was obtained from Bath, Jamaica, where it was collected by Professor Bower. It belongs to a group of species (Nos. 75-9) placed together by Hooker in the Synopsis Filicum, and characterized by 'fronds several feet long, usually climbing'.]

² Gwynne-Vaughan and Kidston: On the Origin of the Adaxially Curved Leaf-trace in the Filicineae. Proc. Roy. Soc. Edin., vol. xxviii, Part vi, 1908, p. 433.

³ Kidston and Gwynne-Vaughan: On the Fossil Osmundaceae, Part IV. Trans. Roy. Soc. Edin., vol. xlvii, Part iii, 1910, p. 469.

⁴ Paul Bertrand: L'étude anatomique des Fougères anciennes et les problèmes qu'elle soulève. Prog. Rei Bot., vol. iv, 1913, p. 182.

Professor C. Eg. Bertrand¹ evolved an extremely neat and ingenious theory to bring the trace of *Lygodium* into line and explain it in terms of the adaxially curved trace.

In order to test this theory and to find out whether the structural features warranted this idea, I investigated very thoroughly the traces of several species of *Lygodium* with confirmatory results, and I was led to examine the leaves of certain climbing species of *Davallia*, the near relations of which possess most typical C-shaped traces, to see whether similarity of habit had led them in the same direction of modification as appears to have been taken by *Lygodium*.

The result appears to me to be a wellnigh complete confirmation in all essentials of Bertrand's theory, with, however, a slight but interesting modification.

[Bertrand and Cornaille in their explanation of the petiolar trace of *Lygodium* started from the 'ternary chain' exhibited by the smaller traces of *Osmunda*. They based their account on the figures of *Loxsona Cunninghami* as given by Gwynne-Vaughan,² and of *Lygodium* as given by Boodle,³ their interpretation of the structure being indicated by the symbolic notation added to the figures which they reproduced.

The change from the C-shaped trace to the type found in *Lygodium* is thus expressed: 'L'accentuation des plis inverses provoquée uniquement par un épaississement des masses du métaxylème, et l'union de ces plis inverses dans la surface de symétrie change complètement le caractère de la trace, elle n'est plus trace osmondéenne, mais bien trace onocléenne, son accentuation a produit les quadruples des *Lygodium*.'⁴

It is clear from comparison of the annotated figures of the traces of *Lygodium* and *Loxsona* given in Bertrand and Cornaille's plate that these investigators regarded the adaxial hooks of the xylem as still constituting part of the condensed xylem strand in *Lygodium*. The modification of Bertrand's theory referred to above is that (as shown by *Davallia fumarioides*) the adaxial hooks exhibit all stages in disappearance before the *Lygodium*-like condition is attained. In connexion with this it may be noted that in *Davallia aculeata* (Gwynne-Vaughan Coll., Slides Nos. 875-8), which has the same habit as *D. fumarioides*, the hooks appear to be absent throughout.

The following facts relating to the vascular system of the petiole and its branches in four species of *Lygodium* are selected from notes which also deal with other features in the anatomy of this genus. They will serve as a restatement of the problem to which the structure of *Davallia fumarioides* affords the key.]

¹ C. Eg. Bertrand et F. Cornaille: Les caractéristiques des traces foliaires osmondéennes et cyathacéennes, exemples, modifications et réductions. Soc. Hist. Nat. d'Autun, 1902.

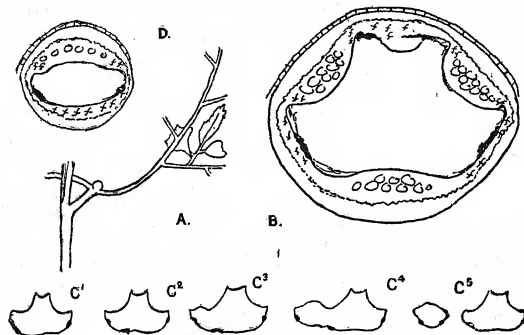
² Ann. of Bot., vol. xv, Pl. iii, Fig. 7, A-E.

³ Ibid., Pl. xix, Fig. 4.

⁴ loc. cit., p. 9.

Lygodium.

LYGODIUM SCANDENS. The petiolar bundle has the well-known peculiar form in transverse section (Text-figure 1, B); the xylem is roughly triangular, the adaxial side being slightly concave. The abaxial median protoxylem is single in young petioles, but in other cases it is apparently double; the lateral protoxylem groups are some distance in front of the lateral prominences. The abaxial protoxylem is apparently exarch, though some evidence was obtained of a mesarch position in young petioles. The protoxylem elements are annular and spiral, and are associated with distinct cavity parenchyma. At the base of the petiole the median protoxylem is not double, and the protoxylem is not crushed and consists of



TEXT-FIG. 1. *Lygodium scandens*. A. Habit sketch. B. Diagrammatic transverse section of petiolar trace. C¹-C⁵. Primary branching of the petiole. D. Branch trace in secondary or tertiary branches. In B and D, and in some of the following text-figures, the protophloem is indicated by a line or dotted line, the sieve-tubes of the metaphloem by small circles, and the incipient phloem-fibres or small thick-walled sieve-tubes by *f*.

small elements which are probably scalariform. It is thus questionable whether there is true protoxylem in this region. Protophloem and phloem are present all round the xylem. The chief development of large sieve-tubes of the metaphloem is in the lateral and adaxial bays. Near the dorsal and lateral prominences of the xylem the small sieve-tubes have thick, soft-looking walls and may be regarded as incipient phloem fibres, the walls of which, however, are never lignified.¹

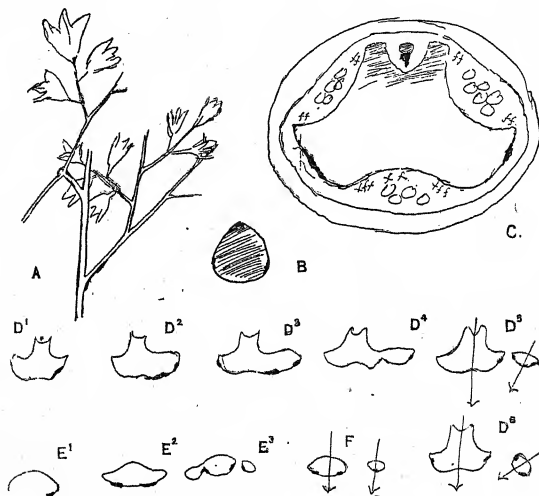
In the origin of a primary branch of the petiole one of the lateral protoxylem groups elongates (Text-fig. 1, C¹) and divides into two (Text-fig. 1, C²). The outer protoxylem is carried out as the protuberance of the

¹ Cf. Boodle, l. c., p. 368.

xylem is formed (Text-fig. 1, C³), and the inner protoxylem again divides (Text-fig. 1, C⁴), giving rise to the proximal lateral protoxylem of the branch, and the protoxylem remaining in the parent axis (Text-fig. 1, C⁴, C⁵). The median protoxylem of the petiolar bundle takes no part in the branching; if there is a true median protoxylem of the primary branch-trace it must arise *de novo*. In other respects the structure of the trace of the primary branch resembles that of the petiolar trace.

The primary branch bears two secondary branches opposite one another and then ends blindly (Text-fig. 1 A).

The origin and structure of the trace to a secondary branch are

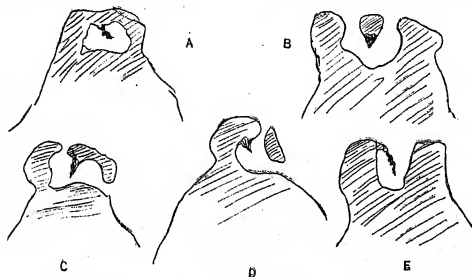


TEXT-FIG. 2. *Lygodium japonicum*. A. Habit sketch. B. Diagrammatic transverse section of xylem strand of petiolar trace at base of petiole. C. Diagrammatic transverse section of petiolar trace in free petiole. D¹-6. Departure of xylem strand to primary branch of the petiole. E¹-3. Same, to secondary branches. F. Same, to tertiary branch.

similar to those of the trace to a primary branch, but the abaxial prominence is less marked. The outline is oval; protophloem and small sieve-tubes surround the xylem. Only one group of large sieve-tubes is present, and lies on the abaxial side; on the adaxial side and also near the lateral ends incipient fibres occur. The median prominence of the xylem is more or less obsolete, and the only protoxylems present are the two adaxial lateral groups (Text-fig. 1, D).

[A photograph of the petiolar trace of *L. scandens* giving off the trace to a primary branch is shown in Plate XIV, Photo 9.]

LYGODIUM JAPONICUM. All branches of the leaf except the lowest terminate in a suppressed growing point, and bear a pair of secondary branches just below the apex (Text-fig. 2, A). The petiolar trace (Text-fig. 2, C; Plate XIV, Photo 10) agrees in general plan of construction with that of *Lygodium scandens*. The lateral protoxylems appear to be quite exarch; they very early become crushed by the surrounding parenchyma. The median protoxylem is single, and in several cases was obviously mesarch with a closed ring of centrifugal metaxylem on its abaxial side (Text-fig. 3, A; Plate XIV, Photo 10). In other cases the persistent centrifugal elements are confined to a small group immediately abaxial to the actual protoxylem and separated by parenchyma from the xylem



TEXT-FIG. 3. *Lygodium japonicum*. Diagrams showing position of abaxial protoxylem.

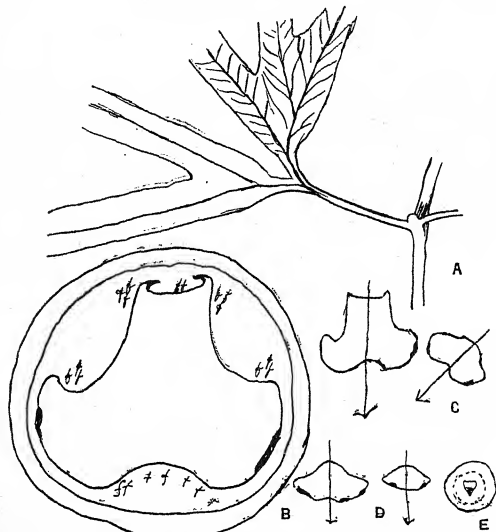
teeth on one or both sides (Text-fig. 3, B, C, D, E). The actual protoxylem elements are very soon crushed by the surrounding cells of the xylem parenchyma, which become somewhat enlarged. Often all the centrifugal tracheae become crushed at the same time as, or shortly after, the actual protoxylem; in this case the extremity of the median lobe is occupied by a little bay of parenchyma, which includes the crushed protoxylem and centrifugal elements and separates the two teeth composed of scalariform elements. In fact the lobe appears to be bifid and to possess two protoxylems.¹ The actual protoxylem is, however, always single, and is usually more or less attached to one of the teeth of small metaxylem elements (Text-fig. 3, D and E).

All the actual protoxylem elements are annular or spiral, but these die out when traced down to the brown basal part of the petiole, although the

¹ Cf. Boodle, l. c., Pl. xix, Fig. 4.

position of each strand of protoxylem is still indicated by a group of small scalariform elements. Towards the very base of the petiole the prominences of the xylem strand become less marked, especially the lateral ones, and the adaxial sinus disappears (Text-fig. 2, B).

The protophloem completely surrounds the xylem, but metaphloem elements only occur in groups on the abaxial flanks and in the adaxial sinus; the sieve-tubes are often in contact with the tracheae. Phloem



TEXT-FIG. 4. *Lygodium dichotomum*. A. Habit sketch. B. Diagrammatic transverse section of petiolar trace. C. Departure of xylem strand to primary branch of petiole. D. Xylem strand at third and fourth dichotomy. E. Trace of vein.

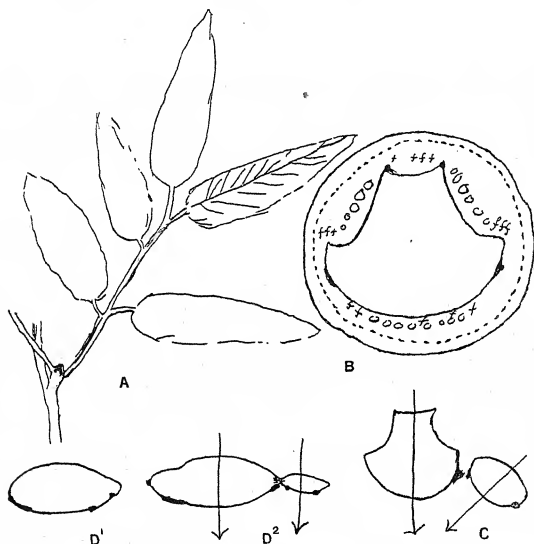
fibres were not seen, but the small sieve-tubes near the prominences are especially thick-walled (Text-fig. 2, C, f).

The pericycle is two-layered opposite the positions of maximum development of the phloem, and one-layered elsewhere.

The branching of the petiole (Text-fig. 2, D¹⁻⁴) essentially resembles that in *L. scandens*. The trace of the primary branch, which has no abaxial protoxylem, swings round (Text-fig. 2, D⁵), so that its median plane is almost at right angles to that of the main trace (Text-fig. 2, D⁶).

In the origin of each of the secondary branches a protoxylem of the primary branch elongates and divides (Text-fig. 2, E², F), supplying a single protoxylem, which doubtless later divides into two, to the secondary trace. In this and higher branchings the plane of the branch is practically parallel to that of the mother axis (Text-fig. 2, E, F).

LYGODIUM DICHOTOMUM. The xylem of the petiolar trace (Text-fig. 4, B) has a deep adaxial indentation so as to make it almost three-lobed.



TEXT-FIG. 5. *Lygodium volubile*. A. Habit sketch. B. Diagrammatic transverse section of petiolar trace. C. Departure of xylem strand to primary branch of petiole. D¹, D². Departure of xylem strand to secondary branch of petiole.

The abaxial projection has a wide sinus, and there are two distinct protoxylems facing one another, one on each tooth. This interpretation is confirmed by the presence of thick-walled sieve-tubes in the sinus in contact with the tracheae of the metaxylem. The position of the two lateral protoxylems and the distribution of the phloem are exactly as in *L. japonicum*.

Branches of the trace of the first (Text-fig. 4, C) and subsequent

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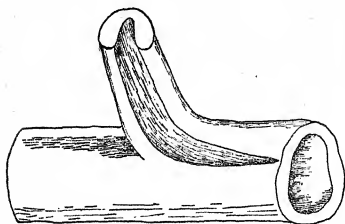
(Text-fig. 4, D) orders have the same structure, and are given off in the same way as in *L. japonicum*, and, as in that species, a dorsal protoxylem is absent in the branch-traces.

LYGODIUM VOLUBILE. The xylem of the petiolar trace (Text-fig. 5, B) has no anterior sinus. The exarch, lateral protoxylems are some distance anterior to the lateral lobes. The posterior protoxylem is double and exarch. The method of branching of the trace (Text-fig. 5, C, D) is essentially similar to that in *L. dichotomum* and *L. japonicum*.

Davallia fumarioides, Swartz.

RHIZOME. The plant has a fairly stout creeping rhizome and shows somewhat frequent dichotomous branching. The very numerous roots arise chiefly, if not exclusively, from the under surface, and show no regular order. The leaves on the upper surface appear to stand in two rows.

One specimen was apparently quite young, the stem being small in the



TEXT-FIG. 6. *Davallia fumarioides*. Diagram of vascular system of rhizome including a node and the base of a leaf-trace.

first formed region and the petioles slender. It seems to have grown at first erect, and later to have bent over, but the erect region seems to have been dorsiventral. Another specimen also showed a narrow juvenile region, but this was horizontal and dorsiventral.

The rhizome is closely beset with narrow brown paleae of very different width, continued above into a single series of cells, and terminating in a pointed non-glandular cell. The larger paleae are fairly wide at the base; others are two or three cells wide for the lower two or three tiers only, and are clearly derived from uniseriate hairs; such simple hairs are also present.

The rhizome contains a well-defined solenostele. In the earlier formed region of the stem the vascular ring is distinctly thicker on the under side than on the upper. In the later formed regions this difference disappears, or at any rate becomes less marked (Text-fig. 6).

The cortex consists of a broad outer sclerotic zone of elongated, rectangular, blunt-ended cells with brown coloured walls. The very narrow inner cortex is made up of two to five layers of cells with thin cellulose walls. The central ground tissue has very thick brown walls, and is also separated from the stele by two or three layers of thin-walled cells.

The endodermis and also the pericycle are similar on the outside and inside of the stele; the pericycle consists of two or three layers.

Phloem is present both externally and internally, but there is proto-phloem only on the outside.

The xylem has abundant xylem parenchyma; the woody cylinder is rather stout, especially on the under side, where it is six to ten tracheae thick. The smallest elements are peripheral, especially near the points of root insertion and on the back of the leaf-trace. These elements are the first differentiated, and may be regarded as an exarch protoxylem; the differentiation of the rest of the xylem is irregular. The tracheae in the stem are all scalariform, even the smallest peripheral ones.

The stem stele branches by dichotomy, the solenostele nipping into two without opening.

ROOTS. The structure of the roots varies considerably; the majority have a normal diarch stele with phloem on each side of the xylem band. In a considerable number, however, the phloem strand is markedly weaker on one side of the xylem than on the other, and the band of xylem is curved, so that the two protoxylems approach one another on the side of the weaker phloem.

In some cases the weaker phloem strand has disappeared, and the curvature of the xylem band is so marked that the protoxylems are almost confluent. In a few cases this seems actually to be the case, and the stele presents a collateral structure and shows an apparently single protoxylem; in fact in these roots a monarch collateral structure has been reached. The collateral structure is mostly found near the point of insertion, whereas farther out the structure becomes more normal.

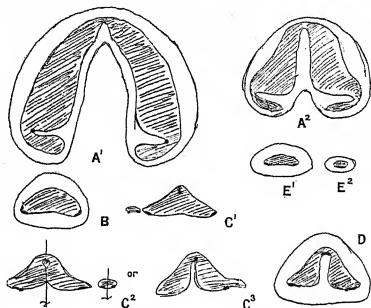
DEPARTURE OF THE LEAF-TRACE. As may be seen from Text-fig. 6, the concavity of the departing leaf-trace faces directly to the median dorsiventral plane of the rhizome. The gap is closed as the apically directed flange of the leaf-trace is given off and does not extend beyond the departure of the leaf-trace.

PETIOLE. The petiole and its main branches are beset with little downwardly curved spinose emergences; these are composed of the somewhat elongated cells of the sclerotic outer cortex, the epidermal cells being also elongated. Within the outer sclerotic cortex of the petiole is a narrow parenchymatous inner cortex. The outer cortex is traversed by transpiration tracts almost to the base of the petiole.

The petiolar trace is at first an open wedge-shaped C (Text-fig. 7, A¹). On passing upwards the concavity gradually closes from the abaxial to the adaxial side (Text-fig. 7, A²; Plate XIV, Photo 1). A small island of one or two cells surrounded by endodermis is often isolated on the adaxial side as the closure proceeds. The xylem strand is hooked, although the trace is not. The hooks are short and stout, with enlarged club-like ends

three to five tracheae thick. The flanks are very stout and have quite distinct external lateral bays. At some distance from the stem three protoxylem groups are present, but these die out before the trace enters the cortex of the stem. The protoxylem strands have usually well-marked groups of cavity parenchyma in front of them. In weaker petioles the xylem of the hooks diminishes till it may be represented by a single series of tracheae.

Within the endodermis of the petiolar trace is a pericycle two or three layers thick on the abaxial and concave surfaces, and three to five layers thick at the sides opposite the bays. The phloem extends all round, but the protophloem is confined to the outer side, being entirely absent in



TEXT-FIG. 7. *Davallia fumarioides*. A¹. Diagrammatic transverse section of petiolar trace at base of free petiole. A². Same, some distance up. B. Trace of primary branch at base of the branch. C¹⁻³. Departure of xylem strand to a secondary branch. D. Primary branch trace above its second branch. E^{1, 2}. Traces in secondary and tertiary branches.

the concavity; the phloem is in greatest quantity opposite the lateral bays and in the bays of the hooks.

When the trace is closed up and has become reniform in transverse section, the middle of the strip of tissue between the halves of the xylem strand consists of pericycle which is often somewhat lignified; this is lined on either side by phloem. Farther up, when the xylem masses of the flanks begin to approach each other, the pericycle disappears, and the centre is occupied by a strip of phloem. Still farther up the phloem is reduced to a single row of sieve-tubes separated from the tracheae on either side by cells of the xylem sheath.

The fact that the petiolar trace of *Davallia fumarioides* appears to be more open in the basal region is not surprising. The climbing habit is not immediately impressed upon the petiole. At first its structure is like that

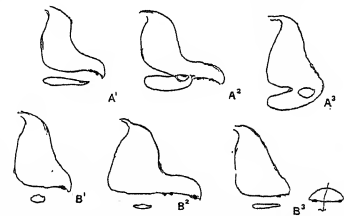
of an ordinary petiole; climbing adaptations would only be developed in this region after some time.

BRANCHING OF THE PETIOLAR TRACE. For a short distance below a branch the hook separates off from the margin of the xylem strand (Text-fig. 8, A¹), and the internal phloem becomes continuous with the outer. The protoxylem divides, and the outer protoxylem moves out on the xylem of the branch-trace, which at the same time elongates laterally. The protoxylem divides again, and a second protoxylem also moves out. Before the branch-trace is free the xylem of the hook joins up with that of the flank on the outside of the remaining protoxylem; it also seems to join up on the inner side of the protoxylem, so that the latter is temporarily immersed (Text-fig. 8, A²); soon, however, about half an inch above, the protoxylem again becomes clearly endarch (cf. Plate XIV, Photos 2, 3).

In weaker leaves the hooks of the xylem strand separate off from the flanks some distance (even as much as one or two inches) before the branch departs (Text-fig. 8, B¹), and after its departure (Text-fig. 8, B²) they do not again join on to the outside of the protoxylem till a long way above; the distance over which the hook remains separate below and above the departure of a branch-trace increases on passing up the rachis. In these weak petioles the hook never joins on to the flank on the inside of the protoxylem, so that the latter is never mesarch (cf. Plate XIV, Photos 4, 5).

The trace of the primary branch, when in the cortex of the rachis, is always very bluntly triangular in outline (Text-fig. 7, B; cf. Plate XIV, Photo 3), and its median plane is parallel to that of the rachis. The xylem has the form of an ellipse. There are only two protoxylem groups; these lie on the adaxial side of the xylem, one at each end of the ellipse. At the base of the free primary branch the trace is the same, but the xylem is more nearly triangular (Plate XIV, Photo 6). At first there is no sign of a dorsal protoxylem, but one begins to appear, either just below the first lateral branch or not until the branch-trace is free. At first it is completely mesarch and near the dorsal surface (Text-fig. 7, C¹); the protoxylem elements may be surrounded by a small island of parenchyma (Text-fig. 7, C²; Plate XIV, Photo 7).

During or after the departure of the second lateral branch the xylem



TEXT-FIG. 8. *Davallia fumarioides*. A¹⁻³. Departure of trace to primary branch of the petiole. B¹⁻³. The same in a weaker leaf.

parenchyma and the phloem on the adaxial side of the xylem of the primary branch encroach on it so as to form a sinus which eventually reaches the protoxylem (Text-fig. 7, C³; Plate XIV, Photo 8). The protoxylem thus becomes endarch, the xylem now having the form of a C; this remains open through subsequent branchings (Text-fig. 7, D). When the trace to a secondary branch is given off, a small xylem strand departs from the end of the arm of the C, and is supplied successively with two protoxylems (Text-fig. 7, C¹⁻³; Plate XIV, Photo 7). The trace of a secondary branch never obtains a dorsal protoxylem (Text-fig. 7, E¹), and the trace to a branch of the next higher order has only one adaxial protoxylem (Text-fig. 7, E²).

SUMMARY.

[1. Bertrand and Cornaille's interpretation of the petiolar trace of *Lygodium*, as derived by the union of the inverse folds of the metaxylem of a C-shaped trace, is confirmed.

2. In *Lygodium japonicum* the dorsal protoxylem of the petiolar trace, consisting of annular or spiral elements, is not exarch but mesarch, being more or less completely enclosed by centrifugal metaxylem.

3. In the petiolar trace and branch-traces of *Davallia fumarioides* all stages between the open C-shaped trace and the condition present in *Lygodium* are found.

4. In this condensation of the C-shaped trace of *Davallia fumarioides* the adaxial hooks of xylem completely disappear. It may be inferred that they are absent in the trace of *Lygodium* also.]

DESCRIPTION OF THE FIGURES IN PLATE XIV.

Illustrating Professor Gwynne-Vaughan's paper on *Some Climbing Davallias and the Petiole of Lygodium*.

(All these figures are from untouched photographs.)

Photo 1. Transverse section of the petiolar trace of *Davallia fumarioides* four inches below the first branches; cf. Text-fig. 7, A¹. Slide No. 2148. (× 46.)

Photo 2. Transverse section of the petiolar trace of a strong leaf of *Davallia fumarioides* just preparing for the departure of the trace to the first primary branch on the left; cf. Text-fig. 8, A. Slide No. 2149. (× 46.)

Photo 3. Section of the same petiolar trace with the trace to the first primary branch just separated on the left; cf. Text-fig. 8, A. Slide No. 2149. (× 46.)

Photos 4, 5. Transverse section of the petiolar trace of a weaker leaf of *Davallia fumarioides* just below the separation of the trace to one of the second lowest pair of primary branches and during the separation of the xylem of the trace; cf. Text-fig. 8, B. Slide No. 2160. (× 46.)

Photo 6. Transverse section of the trace of a primary branch of *Davallia fumarioides* near the base of the branch; cf. Text-fig. 7, B. Slide No. 2154. (× 67.)

Photo 7. Transverse section of the trace of a primary branch of *Davallia fumarioides* which has just given off to the left the trace to the first secondary branch; cf. Text-fig. 7, C². Slide No. 2154. (× 67.)

Photo 8. Transverse section of the trace of a primary branch of *Davallia fumarioides* above the departure of the first pair of secondary branches; cf. Text-fig. 7, C³, D. Slide No. 2164. (× 67.)

Photo 9. *Lygodium scandens*. Transverse section of petiolar trace, showing the trace to the second primary branch going off on the left; cf. Text-fig. 1, C⁴. Slide No. 2073. (× 46.)

Photo 10. *Lygodium japonicum*. Transverse section of petiolar trace, showing the abaxial protoxylem enclosed by centrifugal metaxylem; cf. Text-figs. 2 C, 3 A. Slide No. 2095. (× 67.)

The general resemblance between the petiolar trace and the origin of a primary branch-trace in *Davallia fumarioides* and in *Lygodium scandens* will be evident on comparison of Photos 3 and 9, while a still closer similarity can be traced between the petiolar trace of *Lygodium japonicum* (Photo 10) and a particular stage of the primary branch-trace of *Davallia fumarioides* (Photo 7). These pairs of figures have been placed together on the plate to facilitate comparison.

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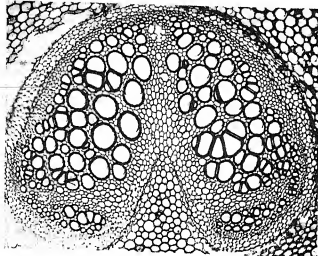
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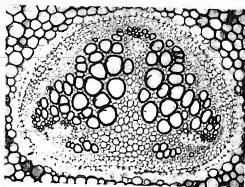
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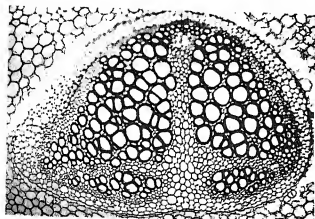




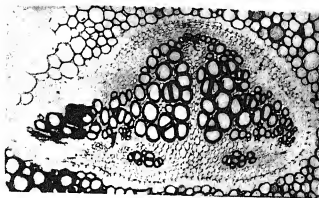
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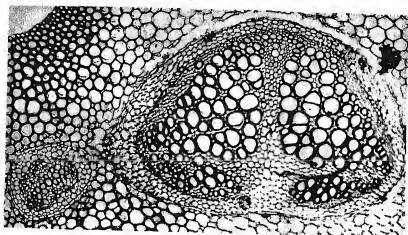
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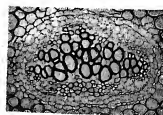
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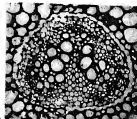
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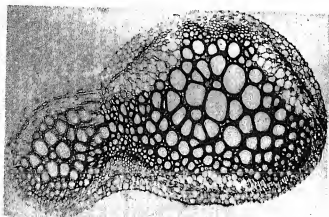
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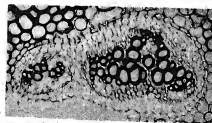
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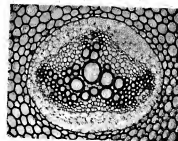
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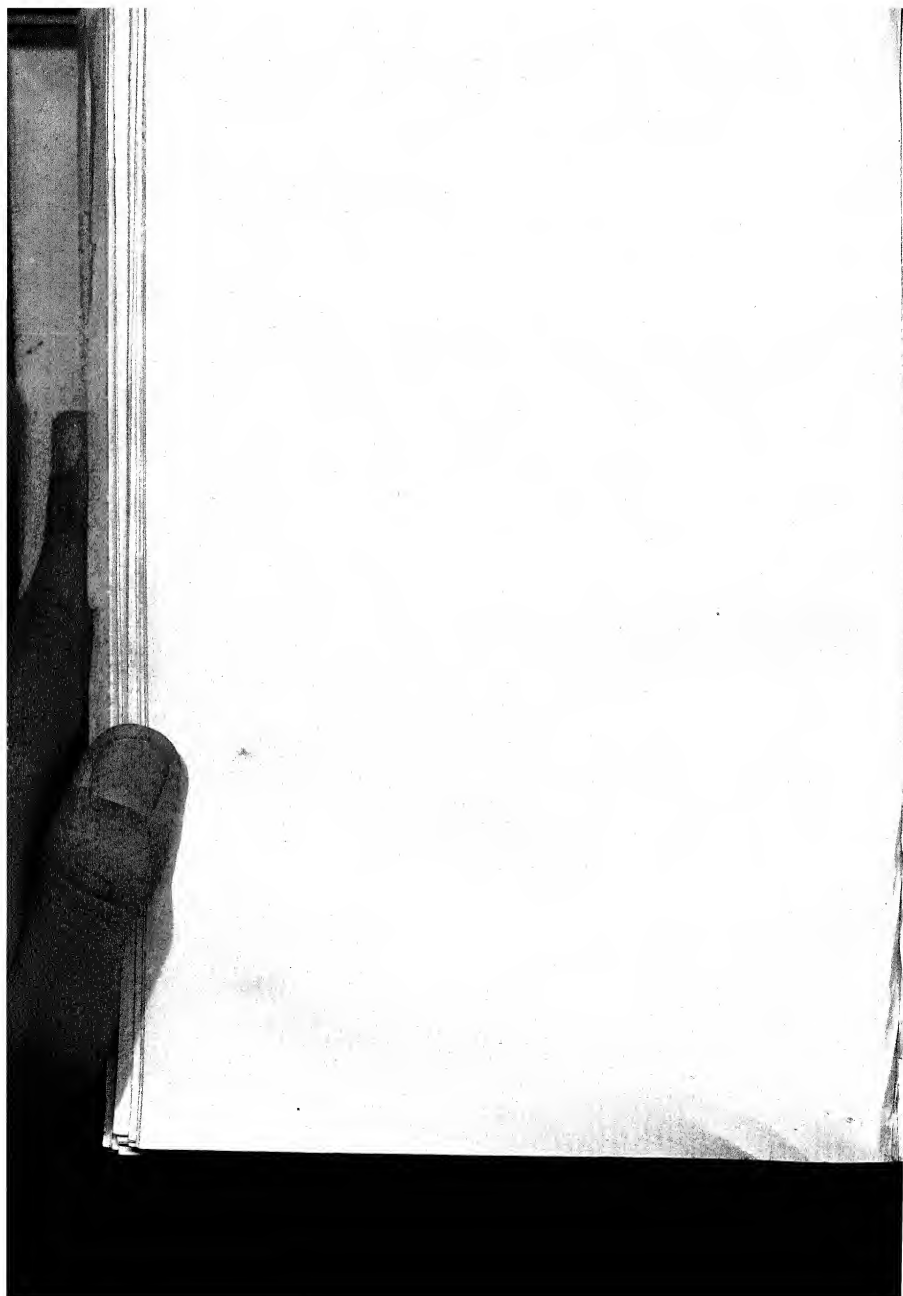
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The Morphology of the Monocotyledonous Embryo and of that of the Grass in particular.

BY

W. C. WORSDELL, F.L.S.

With ten Figures in the Text.

ABNORMAL COLEOPTILE.

SOME seedlings of *Zea Mais* were kindly placed at my disposal by Dr. E. J. Salisbury, in a number of which (he himself has observed ten in all) the coleoptile was forked, or divided into two equal parts for a short

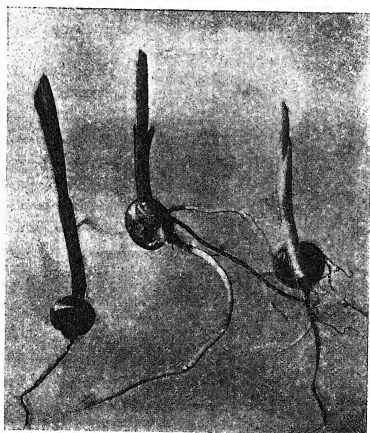


FIG. 1. *Zea Mais* (Maize). Seedlings attached to grain, showing the forked coleoptile and the plumule grown beyond it. E. J. Salisbury photo. Slightly reduced.

distance from the apex (Figs. 1 and 2). The remaining organs of the seedlings were quite normal. I have myself raised a number of seedlings (from seed supplied by Dr. Salisbury) which show the same feature of the coleoptile.

[Annals of Botany, Vol. XXX. No. CXX. October, 1916.]

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As the above would seem to be an uncommon abnormality, it seems desirable to indicate what, in my opinion, is its probable meaning. This involves a brief discussion of the much-debated morphology of the different parts of the Grass-embryo, as also of the Monocotyledonous embryo in general. Every one is familiar with the structure of the Grass-embryo, so

it need not here be described in detail. I will merely take each of the main parts in turn and give a brief historical account (a complete one is unnecessary, having been given by previous writers) of the views held as to their nature.

NATURE OF THE SCUTELLUM AND COLEOPTILE.

The *scutellum* is the hypogeal absorptive organ. Regel, Hofmeister, and Gris regarded it as of axial nature; Agardh as the tegument of the endosperm. The majority have held it to be either an entire cotyledon or part of one. Treviranus, Bischoff, Demoor, Le Maout et Decaisne, Hackel, Warming, Bruns, Coulter, belong to the first group. Those who regard scutellum and coleoptile (or plumular sheath) as together constituting the cotyledon include Mirbel, Cassini, Raspail, Bernhardt, Klebs, Schlickum, Hanstein, Hegelmaier, Fleischer, Celakovsky, van Tieghem. In most of these last cases the scutellum is described as the lamina of the cotyledon, and the coleoptile as the sheathing portion, or else the ligule, or a pair of fused stipules.

Of these various views as to the nature of the scutellum, the last one, to which the majority subscribe, is almost certainly the correct one. Hanstein's excellent researches into the development have clearly demonstrated this, as both his description and figures indicate. He says: 'The earliest origin of the upper circular protective rim from a forwardly and downwardly directed outgrowth of the already differentiated first leaf unmistakably shows it to be a sheathing portion of the latter. This is confirmed by its subsequent growth around the anterior and lower side of

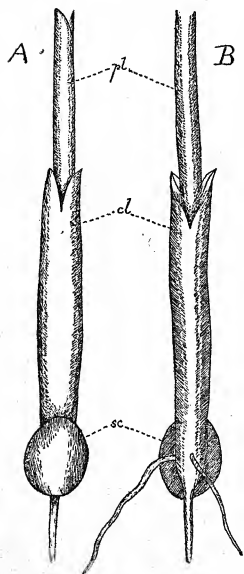


FIG. 2. *Zea Mays*. Seedlings with the grain removed, seen from the dorsal (A) and ventral (B) sides, showing the radicle, adventitious roots, scutellum (*sc*), forked coleoptile (*cl*), and plumule (*pl*).

the bud. It cannot therefore be regarded as an independent phyllome, for its main portion on the posterior side, judging by the plastic processes of its construction, arises, not from a shoot, but from an older leaf.' That is to say, the coleoptile clearly arises, in the early stages of the ontogeny, as part and parcel of the scutellum. This is illustrated by Fig. 3 (A-E), taken from Celakovsky's paper, and whose description of which I here quote. A shows the earliest stage in which the ligular outgrowth (here directed downwards) and the scutellum are clearly parts of one organ. 'In B the ligule is more developed and the angle between it and the scutellum has deepened, a is further separated from c , and the angle abc is more obtuse than in A. In C abc has become very obtuse, so that the ligule is only slightly connected with the scutellum. In D the growth occurring towards the plumule has caused b to fall into the line ac , whereby all connexion between the scutellum and the ligular sheath is lost. In E, owing to the continued extension in the hypocotyl or in the cotyledonary node, a has

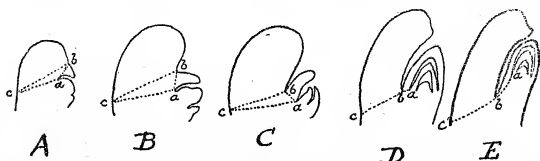


FIG. 3. A-E, Grass-cotyledon, showing successive developmental stages. ac = limit between cotyledon and hypocotyl; a , the angle made by cotyledonary sheath with plumule; b , the angle separating the ligular outgrowth from the scutellum in A, B. (From Celakovsky after Hanstein.)

become carried farther from b and c in the same direction, i.e. the base of the sheath becomes stretched along with the node, giving rise to the mesocotyl (ab).

'As a result of all these processes, the limit between leaf and axis continually changes. The cell-tissue, which in A-C is included in the dotted triangle, there occurs above the insertion of, and belongs to, the cotyledon; in D the same tissue, increased in amount by growth, has come to form part of the hypocotyledonary node, and when a mesocotyl is formed and elongates, the outer basal tissue of the sheath forms part of the mesocotyl and constitutes the outer foliar-base of the sheath.' It is thus seen how the sheath and the scutellum (lamina of cotyledon) become separated from one another so widely, losing every trace of connexion.

Hegelmaier also concluded, from his study of the development of *Triticum vulgare*, that, scutellum and coleoptile together constitute the cotyledon.

Great importance may be attached to these developmental data of the embryo. It is to them we should turn for light on the morphology

of the parts concerned, and not to the later stages. In many Grasses the older condition is marked by the appearance, as above referred to, of the 'mesocotyl', i.e. an internode-like area becomes developed between the scutellum and the coleoptile. In some, e.g. *Zizania*, *Leersia*, *Oryza*, the mesocotyl is of very considerable length, and gives the exact impression of an internode (Fig. 4). Mere appearances, however, must not mislead us in this case. The early developmental history clearly shows that the coleoptile is part of the cotyledon (scutellum). Van Tieghem's anatomical

researches showed that the mesocotyl was not an internode, but the first, abnormally extended node. Schlickum also, by the same method, concluded that the mesocotyl possesses hypocotyledonary structure (Fig. 5). Sandeen found that the very long mesocotyl of *Panicum* has the same structure as an adventitious root. 'It is only necessary to read the papers of the two first-named authors, containing a record of exact observations into the anatomical structure of the mesocotyl, in order to see that, whether it belong to the node or the hypocotyl, it cannot, in any case, belong to the epicotyledonary region. Celakovsky points out that inasmuch as the coleoptile is proved by the developmental history to be part of the scutellum, the mesocotyl must therefore represent a much-extended node. The anatomy supports this. There remain over no other valid reasons, save those resting on mere appearances, for regarding it in any other light. What has really occurred during the elongation of the node is that the base of the coleoptile has become congenitally concrescent therewith, the 'carrying-up' of the sheath being due to this fact, just as the 'carrying-up' of a bract on a peduncle is due to congenital fusion of its basal region with that organ. A perfectly analogous and parallel case to the mesocotyl of the Grass-embryo is, as Celakovsky points out, to be seen in the axial stipular which separates the leaf-stalk of



FIG. 4. *Zizania aquatica*. Longitudinal section of embryo showing the scutellum (*sc*), epiblast (*e*), the elongated mesocotyl (*m*), and the coleoptile (*cl*). (After Bruns.)

Ficus elastica from its ochreate stipular sheath, and which is doubtless due to the same cause.

Bruns's arguments in support of the internodal character of the mesocotyl are easily refuted in the light of the known facts: his conclusions are based solely on the mere appearance presented by the mature embryo, without any reference to the important facts of the development.

Coulter has recently given vigorous support to the same theory as that held by Bruns, viz. that the mesocotyl is the first internode of the epicotyl, thus bringing his view that the epiblast is a second cotyledon into line with the rest of the morphology. This article is an astonishing one for two reasons. Firstly, because his deductions are based, like those of Bruns, entirely on the outward appearance of the advanced embryo; the mesocotyl looks like an internode succeeding two apparent cotyledons, therefore it must be an internode! Secondly, the important developmental and anatomical facts disclosed by Hanstein, van Tieghem, and Schlickum are completely ignored; the article by this last author and the very important one by Celakovsky are not cited, the entire treatment of the subject being thus one-sided. It seems to me a pity that the writings of these previous workers should have been overlooked, especially as the conclusions involved are rather important.

Schlickum, as a result of his investigations, finds that the Grass-seedling essentially resembles in all its morphological parts that of other Monocotyledons, and a continuous series of transitional forms between the two can be instituted. He says that the coleoptile differs in no essential point from the cotyle-

dinary sheath of other Monocotyledons, such as *Canna* and *Carex*. Just as in the case of other investigated Monocotyledons, there exists a great difference, e.g. in *Oryza* and *Panicum*, between the structure of the coleoptile and that of the first plumular leaf-sheath, whilst, on the other hand, the first and second plumular leaf-sheaths exhibit only trivial differences between themselves. He states further that 'as the rudiment of the coleoptile arises in the tissue complex which is becoming the scutellum, I must, as does also Hegelmaier, agree with Hanstein, and like him, on the basis of developmental data, equate the scutellum with the haustorium and the coleoptile with the cotyledonary sheath of other Monocotyledons'.

From what has been stated above there is obviously no foundation for Bruns's and Coulter's view that the mesocotyl is the first internode of the epicotyl and that the coleoptile is the first plumular leaf. The possession by the coleoptile of two widely-separated vascular strands which are situated much nearer to the two margins than they are to each other, strongly suggests a ligular structure formed by the union of stipules. If this organ represented an independent (first plumular) leaf this type of venation would

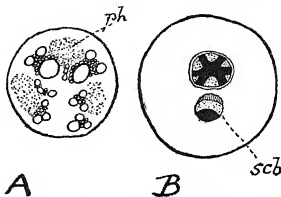


FIG. 5. A. *Panicum miliaceum*. Transverse section of vascular system of mesocotyl. *ph* = phloem (diagrammatic). B. *Oryza sativa*. Ditto. *scb* = bundle destined for scutellum. (After Schlickum.)

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certainly not occur, but, instead, there would be two or three veins placed at equal distances from each other and from the margins. This ligular (stipular) character is further suggested by the seedlings observed by me with a forked coleoptile, this forking being a most natural occurrence in a leaf with this venation, and representing, in my opinion, a partial reversion to the primitive stipular condition of the coleoptile. Regel mentions the interesting and, in this connexion, important fact that the ligule of the foliage-leaf of *Festuca spadiacea* is bifid. *Ammophila* and *Bromus* also have bifid ligules. The position, viz. on the same side as, and opposed to the cotyledon, as also (as above stated) the mode of development, of the coleoptile (especially in those Grasses which, like the Maize, are devoid of a mesocotyl) are still further features in support of its homology with the ligule of the foliage-leaf in this order. How much better it is to trust to these comparative data than to those exhibited by the advanced embryo considered by itself.

NATURE OF THE EPIBLAST.

Turning now to the vexed question as to the nature of the 'epiblast', we find that Poiteau, Mirbel, Turpin, Hackel, Warming, Bruns, van Tieghem, and Coulter regard it as a second cotyledon. This is owing chiefly to its position opposite the supposed lateral main cotyledon or scutellum and at the base of the supposed internode (mesocotyl). It appears widely separated from, and without connexion with, the scutellum, and as it occupies the same relative position as the latter on the opposite side of the primary node, it is best regarded, according to this view, as a second independent leaf or cotyledon.

In my opinion, it will not do to rely, as van Tieghem does, solely on the course of vascular strands for determining the nature of an organ. The conclusions of Bruns and Coulter, again, are based solely on superficial appearances. It is simply astounding that no deeper investigations into the comparative morphology of the organ concerned, nor into the results of researches of other authors in this connexion, have been thought necessary. Undoubtedly, if we are to judge by the *appearance* presented by such embryos as those of *Zizania* (Fig. 4), *Oryza*, and *Leersia*, the epiblast and scutellum are two lateral cotyledons, the mesocotyl is the first epicotyledonary internode, and the coleoptile is the first plumular leaf situated in its proper position (following the distichous arrangement) on the same side as, and directly over, the scutellum. Coulter does not seem to be aware of the existence of Celakovsky's able paper in which quite another side of this question is presented. Therein is to be found a comprehensive and most interesting discussion on the nature of the epiblast. As Coulter has passed it over, I will here give its gist.

Gärtner's view of the epiblast: 'lacinula e scutello oriunda', and van Tieghem's former view: 'une dépendance des bords de l'écusson' [scutellum], are really correct. Celakovsky adopts the natural and reasonable method of comparing the cotyledon with the foliage-leaf. Surely there could be no better plan than that! As we have above seen, the developmental facts and anatomical structure of the mesocotyl show that the scutellum corresponds to the lamina of the foliage-leaf and the coleoptile to the ligule, the sheath of the foliage-leaf not being represented in the cotyledon except at the very earliest stage of all. The fact that scutellum and coleoptile are parts of the cotyledon is in itself sufficient to dispose of the idea that the epiblast represents a second cotyledon. The true nature of the epiblast is revealed by the following facts and deductions. The foliage-leaves of *Hordeum* (Fig. 6), *Triticum*, *Secale*, *Lolium*, and the larger leaves of *Oryza* possess peculiar sickle-shaped appendages to the base of the lamina. If these appendages were to become united on the opposite side of the axis, a structure would result comparable to the epiblast. This last is, however, in many cases (not in all) quite separate and distinct from the scutellum,¹ existing as an independent outgrowth on the opposite side of the axis. Celakovsky found, however, in certain robust leaves of *Oryza* that the appendages were completely separated from the leaf-blade,¹ more linear or lanceolate in shape, hardly curved, directed upwards, and provided with long, bristle-like cilia on the edge nearest that of the leaf-blade. Now if the leaf-sheath and ligule were closed structures (as occurs in species of *Melica*) then the distal margins of the two appendages would, like those of the sheath and the ligule, become united, and a single appendage would result, situated opposite the leaf. Such a condition of things is realized in the seedling of *Oryza*, where the epiblast corresponds to the single appendage. The cause of the marked independence of scutellum and epiblast in many Grasses is that the latter, owing to the disappearance of the sheathing-base in the cotyledon, arises directly from the hypocotyl, so that its original connexion with the cotyledon could easily become obscured.

Further light is thrown on the origin of the epiblast by the contemplation of that of *Stipa*, which is deeply bifid into two equal parts (Fig. 7, A), at once suggesting its composition from two originally separate organs.

¹ As in the case of the mesocotyl, so also in that of the area separating the scutellum-base from the epiblast, and the leaf-blade from the appendages, we can postulate a congenital fusion of the foliar parts concerned with the axis.

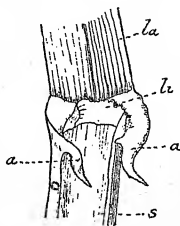


FIG. 6. *Hordeum vulgare*. Base of lamina (*la*) of foliage-leaf, showing auricles (*a*). *li* = ligule; *s* = sheath. (After Celakovsky.)

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The epiblasts of *Koeleria*, *Eleusine* (Fig. 7, B), *Danthonia*, and *Brachypodium* show a similar structure. How Coulter can explain the structure of the epiblasts in these Grasses (figures of which are given by his favourite author Bruns) in favour of the latter being a second cotyledon is to me a mystery. Just as the forked coleoptile observed in certain seedlings of Maize is evidence of its compound nature, in the same way the bifid epiblast of *Stipa* and *Koeleria* is evidence of the compound nature of this organ.

Thus we see that counterparts of all the foliar structures of the seedling can be found for the searching in the foliar structures of the mature plant; and, as a result of a careful comparative investigation, Celakovsky

reaches the convincing conclusion that the epiblast also is part and parcel of the cotyledon, and that there are no natural or legitimate grounds whatever for regarding it as an independent foliar organ.

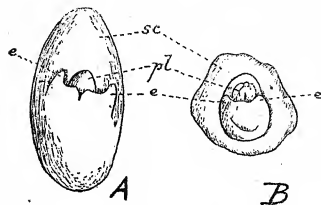


FIG. 7. A. *Stipa arenaria*. B. *Eleusine coracana*. Embryos showing bifid epiblasts (e); sc. = scutellum; pl. = plumule. (After Bruns.)

has a similar outgrowth which is to be regarded as the foliar base of that organ, analogous to the similar outgrowth from the succulent leaf of some species of *Sedum*. The epiblast having become a quasi-independent foliar organ, it has come to form its own foliar base.

POSITION OF THE COTYLEDON.

Finally, we have to determine the *position* of the scutellum (cotyledon), whether it is terminal or lateral to the axis of the seedling.

As the construction of the Grass-embryo is essentially the same as that of other Monocotyledonous embryos, what is true of the latter must also be true of the former. The cotyledon must occupy the same position in both. This is an important point to remember. Now the excellent investigations of Hanstein, of Fleischer, and of Hegelmaier have clearly shown that the cotyledon of Monocotyledons is always *terminal*. Speaking of *Funkia*, Hanstein says: 'The cotyledon is laid down as a massive structure as a continuation in the same direction of the hypocotyl, before a trace of the growing-point exists.' Fleischer says of *Leucosium aestivum* that a terminal cotyledon arises from the entire upper portion of the

embryo; the growing-point occurs at the region between the upper and lower halves of the embryo. In *Funcus glaucus* he found that after the terminal cotyledon a succession of sympodially-formed foliage-leaves occurs, each arising out of the sheath of the preceding one, before any other organ exists at all besides these leaves. He states that this mode of development in *Funcus* appears to contain the key to the understanding of the construction of all Monocotyledonous embryos, for there occurs here several times in succession what occurs only once in the first development of the terminal bud of every Monocotyledonous plant. He points out that Strasburger's view that the ontogenetic development of the Monocotyledonous embryo represents a 'later adaptation', due to the cotyledon pressing the stem-apex to one side, is contradicted by the fact that the later cotyledon with the later hypocotyledonary segment constitute a morphological unity, completely corresponding to that of the Archisperms, which later becomes in its extension the shoot; that there is thus not the slightest trace of a lateral sprouting of the cotyledon from a pre-existing axis. 'That the axis exists before the cotyledon cannot be maintained, for it has no growing-point. On the contrary, the cotyledon exists before the commencement of activity of the growing-point. . . . It exists, however, not as a cotyledon, but as part of a thallus, which becomes a cotyledon only after the appearance of activity of a growing-point.'

Hegelmaier also, in *Canna indica*, notes the development of the first three plumular leaves without there being any stem-apex present at all. In *Pistia* he found that seven or eight plumular leaves arose, each out of the base of the preceding, in a spiral sequence, with no sign at all of a stem-apex. He says: 'The clearly terminal position of the cotyledon is merely a single phenomenon in a whole group of such, but one of the most striking of the group, for the following leaves, which are equally with the cotyledon (relatively) terminal, are laid down in somewhat closer approximation to the preceding leaf-apex and thus form a gradual transition to the production of a so-called bud-axis with its own growing-point.' He says that if the theory of the cotyledon assuming the place of an aborted stem-apex be extended, as it ought to be, to the plumular leaves (which arise in the same way as the cotyledon) it would lead to absurdities.

I would draw particular attention to the *sympodial* arrangement of the cotyledon and the first few plumular leaves in the embryos above-mentioned. It involves the complete absence of an epicotyledonary axis and of laterally placed leaves. Hence, neither the cotyledon nor the plumular leaves concerned can be lateral in position, and no evidence can be adduced to show that this is a secondary and derived condition of things.

But the most fundamental evidence for the phylogenetically terminal position of the cotyledon has yet to be given. It rests on the sure and unequivocal basis of embryological data which are common to all the

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divisions of vascular plants. In this connexion I cannot do better than quote Celakovsky :

'The cotyledon of Monocotyledons is, in my opinion, primitively single and terminal. The Monocotyledonous embryo is, before the bud and root are laid down, as Hanstein has stated, a simple thallus. This is completely homologous and equivalent to the sporogonium of Bryophytes in the embryonic state of the latter, as is shown by the similar mode of cell-division. The further construction of the thallus is indeed different from that of the sporogonium, corresponding to the phylogenetic advance from the earliest stage of the Thallophytes to the second stage of the Vascular Plants.' 'The simplest primitive metamorphosis, according to my repeatedly-expressed view, consisted in the development of the upper terminal portion of the embryo (which in the Mosses became the spore-capsule) as a purely vegetative assimilating organ, viz. a leaf, as occurs, according to Kny, in *Ceratopteris* among the Ferns before branching of any kind occurs, and the same thing is repeated in Monocotyledons. Just as the Moss-sporogonium becomes differentiated into two parts—the basal, sterile seta, and the terminal spore-capsule—in the same way is differentiated the embryonic thallus of Monocotyledons into the terminal leaf (cotyledon) and the basal stem-segment (hypocotyl), so that thus the Moss-capsule is phylogenetically homologous to the cotyledon, and the seta, or at any rate its basal portion, to the hypocotyl. In the embryonic thallus of *Ceratopteris* and of Monocotyledons, including the Grasses, the stem-bud arises laterally. . . . The embryonic thallus must be held to be the first segment (Glieder) of the leafy shoot; its hypocotyl represents at a later stage the first stem-segment of the further developed embryonic shoot, and its cotyledon the first leaf of the latter.'

The importance of this comparison of embryological structure in the different main plant-groups has never been adequately realized. The basis of comparison must be a perfectly sound one, as embryos are the least variable of all structures, and thus the most likely of all to reveal ancestral features. Hence, if the embryo of Monocotyledons and that of *Ceratopteris* exhibit the same construction as the sporogonium of Bryophytes, the conclusions deduced therefrom as set forth above are perfectly legitimate. The fact that Bryophytes are so distantly separated, in the genealogical tree of the Vegetable Kingdom, from Pteridophytes and Angiosperms can make not the slightest difference, for the embryo-structures, with their unvarying mode of development, constitute intimate connecting links at every stage.

The view above set forth could only be overthrown if it could be shown that the terminal position of the cotyledon in Monocotyledons and *Ceratopteris* has arisen as a secondary modification of the condition obtaining in Dicotyledons¹ and in other Ferns. There is, however, no real

¹ The supposed two cotyledons of this class are, as Hegelmaier points out, present before there

evidence for such a thing, nor can any logical reason be given for supposing such a modification to have occurred. There is, on the contrary, plenty of evidence, some of which has been given above, for holding the opposite view, viz. that the cotyledon is primitively terminal.

A very important matter has yet to be referred to. It concerns the relative degree of development of the *lamina* and the *sheath* of the cotyledon in Monocotyledons. In the majority the lamina greatly overreaches in development that of the sheath. In the Dioscoreaceae and Commelynaceae, as Celakovsky points out, the state of affairs as described by Solms-Laubach is due to the fact that the sheath has developed at an earlier stage than, and consequently ahead of, the lamina (Fig. 10, A-C); *the apical portion of the embryonic thallus has been used up to form the sheath*, which appears in the form of a circular outgrowth; there is *no shoot-growing-point present until a later stage*; the lamina arises subsequently as a lateral outgrowth of the sheath.

Wherever in Monocotyledons the *appearance* of a second cotyledon is found, it can be traced to a special development of the cotyledonary sheath or of the basal portion of the lamina, which inevitably suggests a peripheral or lateral position of the one or two cotyledons. This it is which has misled Coulter into imagining that in the Grass-embryo either two or one, as the case may be, *lateral* cotyledons are present. The appearance is simply due to the very special development of the lamina of the cotyledon as a haustorium and of its basal region in the form (where present) of the epiblast. The latter organ Celakovsky has shown to correspond to modified appendages of the lamina of the cotyledon; the development shows that the coleoptile is the ligular portion of the cotyledon, and that the whole arises as a single organ, on essentially the same lines as in other Monocotyledons, before any other leaves or any trace of a stem-apex is present. Under these conditions it can be nothing else but *terminal*. Coulter himself states that no stem-tip is present even at quite a late stage. In the absence of a stem how is it possible, I ask, for the cotyledon or cotyledons to be peripheral or lateral? Peripheral or lateral to what? What is this 'peripheral zone' he mentions from which the supposed two cotyledons arise on opposite sides? It must be one of two things: either (1) the earliest stage of the two first leaves (cotyledons) whose bases are united to form a sheathing structure, and whose position, of course, must be lateral to a stem; or (2) it represents a single cotyledon with its well-developed basal portion. Now (1) cannot possibly be the explanation, for at that stage there is a complete absence of any stem to which the cotyledons could be lateral; for it is an absolute impossibility for two distinct cotyledons to exist without any axis to which they are attached. Hence (2) must be the true explanation, is any trace of an epicotyledonary axis; hence they cannot be two lateral cotyledons, but a single bifid terminal one.

and the 'peripheral zone' represents the cotyledon with its well-developed basal portion, and this single cotyledon, in the absence of a stem, must, of course; be terminal to the whole embryo, as the earliest stages of development show it clearly to be. From all which it appears that Coulter's position with regard to this matter is ambiguous and illogical.

It having been deduced from all that has been set forth above that in Monocotyledons (all representatives of which have essentially the same type of embryo- and seedling-construction) the cotyledon is always single and terminal, the appearance of two lateral cotyledons in the Grass being due to the supervention of secondary modifications, the two remaining cases

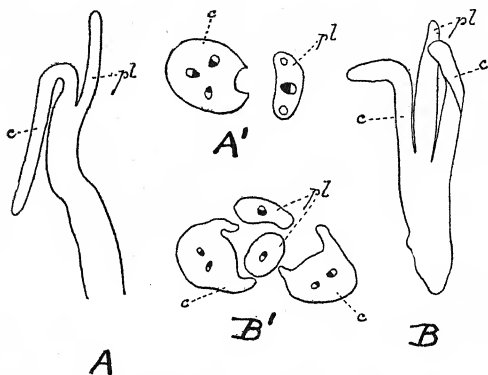


FIG. 8. *Agapanthus umbellatus* (African Lily). A=outline of normal embryo; A'=transverse section of cotyledon (c) and plumular leaf (pl). B=outline of dicotyledonous embryo. B'=transverse section of cotyledons and plumular leaves. (After Coulter and Land.)

which Coulter and Miss Farrell marshal, in the endeavour to demonstrate their thesis of primitive dicotyledony in all Angiosperms, can be easily dealt with. I refer to the embryos of *Cyrtanthus* and *Agapanthus* in which two cotyledons occur. Both cases can be quite well explained as accentuations of the condition met with in the Dioscoreaceae and Commelynaceae in which the development of the sheathing portion of the cotyledon sets in at an early stage, and proceeds to an equal degree with that of the lamina.

On the analogy of the Dicotyledons, if the orthodox view is held with regard to the morphology of the embryo of this class, then it is quite obvious that two cotyledons are present in these cases of *Agapanthus* and *Cyrtanthus*. And it is a most rare and interesting observation to have made that in these cases a second cotyledon is formed by the excessive develop-

ment of the sheath of the other cotyledon. No previous case of the kind has ever been cited, so that the authors have every reason to make the most of it. I say that on the current view their conclusions as to the facts hold good. But the view I prefer to maintain here is that in Dicotyledons, owing to the complete absence, in the majority of cases, of an epicotyledonary axis, two cotyledons cannot possibly be present, but only a single terminal cotyledon which has deeply divided. This argument, therefore, also applies to the cases of *Agapanthus* and *Cyrtanthus*, although in these the appearance of two cotyledons is due to quite another cause, which constitutes the sole importance of the observation.

That no phylogenetic significance attaches to the phenomenon can be deduced from the fact that only a single seedling of *Agapanthus*, according to Coulter and Land's account, possessed two cotyledons (Fig. 8). To suppose that a unique instance, the only example ever known, would be likely to exhibit the character of the ancestors of the whole group is to my mind next door to an impossibility.

If this had been the ancestral feature, instances would certainly be much more numerous, not only in this genus, but in others as well.

The phenomenon must therefore represent a *new*, aberrant departure, of progressive, not reversionary, nature.

The same argument applies to *Cyrtanthus*; although the two cotyledons may occur as a normal feature of the genus (Fig. 9), yet the features of a single genus cannot be taken as indicative of the characters, whether modern or ancestral or both, of the whole class; they are much more likely to be progressive and novel.

That the embryos of one or two members of a modified order like the Amaryllidaceae, with its inferior ovary and other idiosyncrasies, would exhibit more ancestral characters than those of members of less modified orders is in the highest degree improbable. They are more likely, in agreement with the other advanced characters of the order, to show a progressive type of construction.

One may securely conclude, therefore, on all these grounds that the extra structure, opposed to the cotyledon, in these two genera is without doubt a second cotyledon, the result of the very exceptional development of

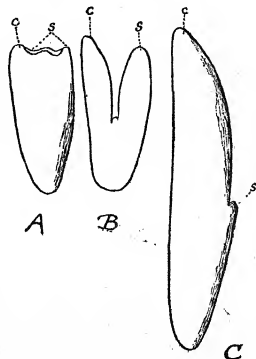


FIG. 9. *Cyrtanthus sanguineus*. A. Exterior view of young embryo. B. Longitudinal section, through the centre of A. C. Exterior view of an older embryo. c = lamina of cotyledon; s = sheath of cotyledon. (After Miss Farrell.)

the sheath of the first one; but that the theoretical deductions drawn from this fact by Coulter and Land and Miss Farrell cannot in any way be accepted.

The same conclusions may be drawn with regard to the embryo of Dioscoreaceae and Commelynaceae (Fig. 10); it may be regarded as exhibiting a progressive feature; the former order is certainly a very

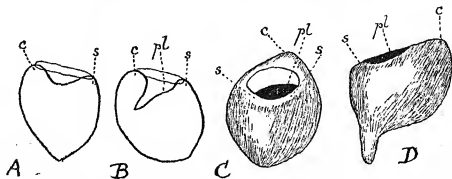


FIG. 10. A and B. *Helorachia* (Commelynaceae). Outline of young embryo (plumular axis completely absent). C. *Thunbergia* (Commelynaceae). Young embryo. D. *Tamus communis* (Dioscoreaceae). Young embryo. c=cotyledonary lamina; s=sheath; pl=indication of plumule. (After Solms-Laubach.)

specialized one, and as regards the latter it is in several ways more specialized than the closely allied order Liliaceae, which is more typical of Monocotyledons generally.

CONCLUSIONS.

1. The *scutellum* is the lamina of the cotyledon, corresponding to that of the foliage-leaf of the Grass. That part of the cotyledon which corresponds to the *sheath* of the foliage-leaf is only present at an early stage of development, and later becomes completely obscured.

2. The *coleoptile* is part of the cotyledon, viz. that which is represented in the foliage-leaf by the *ligule*; this is clearly demonstrated by the early developmental stages of the embryo. The vascular anatomy and the abnormal forking strongly suggest a ligular structure.

3. The *epiblast* is part of the cotyledon, corresponding, as deduced by means of comparative morphological treatment, to the *auricles* of the base of the lamina of the foliage-leaf in certain Grasses.

4. The cotyledon of the Grass differs in no *essential* feature, either as regards its development or morphological construction, from that of other Monocotyledons.

5. The *mesocotyl*, as shown by the above facts with regard to the coleoptile, and by its anatomical structure, is the elongated primary node.

6. The *position* of the cotyledon in all Monocotyledons, as shown by the facts of development, there being no epicotyledonary axis present

on its first formation, is always *terminal*, and is the natural continuation and termination of the hypocotyl.

7. The balance of development of the cotyledonary lamina and sheath may vary in favour of the latter in certain cases, and at certain stages of the ontogeny, as in Dioscoreaceae and Commelynaceae.

8. In certain instances, as in a seedling of *Agapanthus* and in *Cyrtanthus* (both belonging to the Amaryllidaceae), the sheath may develop, at one stage or another, into a second cotyledon. This is not an ancestral, reversionary character, but a novel and progressive one.

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22 JAN 1917

Variations in *Anemone nemorosa*.

BY

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With three Figures in the Text.

FOR several years past the writer has been engaged in the study of Hertfordshire woodlands in which the Wood Anemone is a conspicuous and abundant member of the ground flora. An exceptional opportunity has therefore been afforded of studying the variation to which this species is subject, two striking forms having been encountered.

The type form of the species shows great variation in the degree of hairiness and width of the involucre segments, a statement that is also true of the foliage of the non-flowering shoots. As has been shown by Yule ('Variation in the Number of Sepals of *Anemone nemorosa*, 'Biometrika, 1902) the number of perianth-segments is by no means constant, ranging from five to ten. In general, however, the most prevalent condition is a perianth of six members in two alternating whorls. Two extreme shapes of perianth-segments can be recognized, both of which agree in the fact that they taper towards the apex and attain their maximum width *below* the middle. In the one form the perianth-members are narrow, more or less lanceolate, and taper very pronouncedly both towards the base and apex, so that the adjacent members, except at the base, scarcely overlap. This flower-type is usually found associated with narrow-leaf and involucre segments. At the other extreme the perianth-segments are broader, ovate in form, and with a rounded base. The flower of this latter type is, as a whole, consequently much more cup-like in appearance, due to the greater overlap of adjacent perianth-members. With this type is usually associated an involucre in which the segments are much broader.

In both types the crenulations of the perianth-margin may be obscure or very pronounced. The indentation of the margin may indeed be regarded as an undeveloped form of lobing, and such a view is in harmony with the occasional record of specimens in which the perianth-segments assume a definitely laciniate form (cf. Pryor's 'Flora of Hertfordshire',

p. 2, London, 1887). One specimen of *Anemone nemorosa* which the writer transferred to his garden produced for several years flowers in which one or more members of the outer whorl of the perianth were green and lobed in a manner similar to that of the involucral leaves (Fig. 1). In colour the flowers exhibit all gradations from white to purple, but the deep-coloured forms appear to be definitely associated with certain localities (var. *purpurea*, D.C., Fl. Fr., ed. 3, vol. iv, p. 635, 1815). Var. *caerulea*, D.C. (loc. cit.), with pale blue flowers has not been met with.



FIG. 1. Perianth segment of abnormal flower. The green parts are shown shaded.



FIG. 2. *Anemone nemorosa*, var. *robusta*. $\times 3$.

Two varieties have been encountered in Hertfordshire which from their constant and distinctive characters appear to merit detailed description. For the first of these we propose the name of var. *robusta* (Fig. 2), in virtue of its most salient feature, namely the large size of its parts. Up to the present this has been encountered in one locality only, namely Stocking's Wood, near Harpenden, where it grows in association with the normal type. The characters of this variety are tabulated below, side by side with the corresponding ones of the largest specimens obtainable from the same locality of the normal type, which we can distinguish as var. *genuina*.

	<i>A. nemorosa</i> , var. <i>gemma</i> .	<i>A. nemorosa</i> , var. <i>robusta</i> .
<i>Vegetative organs</i> —		
Colour	Green.	Pale green.
Average diameter of rhizome	4 mm.	5.5 mm.
Average spread of involucre	100 mm.	125 mm.
Width of sheathing-base of involucral leaves	3-3.5 mm.	5-7 mm.
Diameter of inflorescence axis	2 mm.	3.5 mm.
Leaves	Under surface dull.	Under surface glossy.
Hairs	Sparse to numerous.	Numerous.
<i>Reproductive organs</i> —		
Diameter of flower	To 40 mm.	40 mm.
Length of petals	19-20 mm.	18-19 mm.
Maximum width of petals	8-11 mm.	11-12 mm.
Form of petals	Tapering towards apex. Rounded or tapering towards base. Broadest below middle.	Rounded at apex. Rounded at base. Broadest above middle.
Margin of petals	Slightly crenate.	Slightly crenate.
Average length of anthers	1 mm.	1.5 mm.
Carpels	Pubescent.	Densely pubescent.

It will be seen from this comparison that, quite apart from the more robust character of the vegetative organs (in which respect this variety agrees with *γ grandiflora* of Rouy et Foucaud, 'Fl. de France,' p. 44, 1893), the flowers are distinguished from those of the common form by the fact

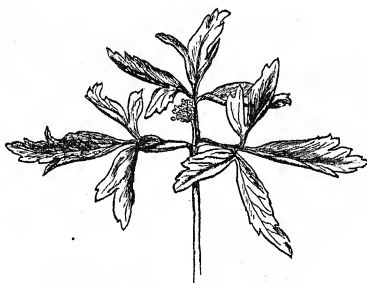


FIG. 3. *Anemone nemorosa*, var. *apetalata*. $\times \frac{1}{3}$.

that the perianth-segments are broadest above the middle and are rounded towards the apex. Rouy et Foucaud (loc. cit.) do not state the form of the perianth-segments in var. *grandiflora*, but the large size of the flowers of this variety, up to 7 cm., would appear to indicate that our variety is distinct.

A second type, which appears to be worthy of varietal rank, usually bears inconspicuous flowers and may be termed var. *apetalata* (Fig. 3). This bears much the same relation to the normal form as *Ranunculus auricomus*, var. *depauperata*, to *Ranunculus auricomus* itself. It is interesting

to note that in the case of both these species the imperfect-flowered variety is usually associated with the more deeply shaded situations. However, the fact that the apetalous character is maintained after the wood in which this variety grows has been coppiced indicates that the characters of its floral organs are not a mere effect of inadequate illumination. The perianth-segments are usually small, six in number, and arranged in two alternating whorls. Most commonly all six are small purplish-green structures of which the outer are the larger (3 to 4 mm. long) and the inner slightly smaller (2 to 3 mm. long). Rarely one to three of the outer segments may be white and petaloid, but the maximum size which these have been observed to attain is 8 mm. in length by 5 mm. in width. In only one instance has a flower of this variety been found in which the inner whorl exhibited petaloidy, and in that case one member only was involved. This variety has been observed in several of the Oak-Hornbeam woods of Hertfordshire, and I have in my possession specimens given me by Mr. W. C. Worsdell which grew at Carnforth, Westmorland.

A short diagnosis of the varieties mentioned is appended:

Var. *genuina*: Phyllis perigonii elliptico-lanceolatis aut ovatis, apicibus acutis, latitudine maxima infra medium. Lateribus inferioribus foliorum non nitentibus. Long. phyll. perig., 19-20.

Var. *robusta*: Phyllis perigonii oblongo-lanceolatis, apicibus obtusis, latitudine maxima supra medium. Lateribus inferioribus foliorum nitentibus. Maior et minus viridis quam var. *genuina* est. Long. phyll. perig., 18-19 mm.

Var. *apetala*: Phyllis perigonii parvis purpurascensibus, vel 1-3 externis albis, petaloideis. Long. phyll. ext., 3-4 mm.; long. phyll. int., 2-3 mm.

Pityostrobus macrocephalus, L. and H. A Tertiary Cone showing Ovular Structures.

BY

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Late Scholar of Queens' College, Cambridge.

With Plate XV and two Figures in the Text.

INTRODUCTION.

ALTHOUGH a number of cones of Abietinean affinity have been recorded from Cretaceous and Tertiary rocks, in almost all cases the internal organization of the ovules has been passed over without comment. This is, of course, partly due to unfavourable conditions of preservation, but even where slides showing a considerable amount of anatomical detail have been in existence for a long time, the older writers have generally contented themselves with a brief description of the main morphological features.

The material which forms the basis of the present description consists in the first place of a number of slides belonging to the British Museum. All but one of these are known to have been prepared from two cones which form part of the Cowderoy Collection bequeathed to the British Museum in 1852. Up to now these two cones have been regarded as representatives of two distinct species, but, as will be seen, examination of the internal structure seems to afford little justification for this view. The larger cone, *Pinites macrocephalus* of Carruthers, has furnished only thick longitudinal sections, while from the other specimen have been cut two transverse sections and two thin longitudinal sections of a small portion of the base. A third transverse section is not definitely referred to either of the above cones, but it agrees in all details with *P. ovatus*, and has probably been cut from the same cone as the others.

I should like here to record my great indebtedness to Professor Seward, who placed the slides at my disposal and who has throughout given me much valuable assistance and advice. Further, owing to the kindness of Dr. Arber I received permission to have sections cut from a specimen of *P. macrocephalus* which was presented to the Sedgwick Museum, Cambridge, some years ago by Mr. C. H. Edgell. With the help of these slides (two

longitudinal and one transverse) a closer comparison of the two forms of cone has been rendered possible. My thanks are also due to Mr. Irwin Lynch, Curator of the Cambridge Botanic Garden, who has granted me facilities in obtaining specimens of recent cones.

It will be convenient in the following description, for the purpose of reference to the different slides, to retain the specific designations, although this can be dispensed with in the general summary. Throughout the paper the generic name *Pityostrobus* will be used instead of *Pinites*, except in the case of citations from particular authors. Nathorst's name *Pityostrobus* is adopted in preference to Feistmantel's term *Pinostrobus*, recently revived by Dr. Stopes, on the ground that the former designation is more appropriate for Abietinean cones which cannot be safely referred to any one recent genus. In the present case the characters clearly point to an affinity with *Pinus*, but nothing is known of the vegetative features of the tree on which the cone was borne.

LITERATURE.

The history of these cones shows many vicissitudes with regard to terminology as well as error in the determination of their geological horizon. Of the four specimens which have been referred to by Carruthers as *Pinites macrocephalus*, the one on which is based the original description of Professor Henslow in the Fossil Flora of Lindley and Hutton (7) is a large cone which was found in clearing out a pond near Dover. Both Lindley and Hutton agreed in assigning the fossil to the genus *Zamia*, and they gave it the name *Zamia macrocephala*. From one of Henslow's figures Endlicher (4) was led to believe that the cone differed from *Zamia* in possessing only a single ovule on each scale, and he therefore established the new genus *Zamiostrobus* for its reception, retaining the specific designation *macrocephala*. Morris (10), in his revision of the fossil Cycadeae, adopts the genus *Zamites* introduced by Presl (11) and includes under it three species of *Zamia* of Lindley and Hutton, among which is *Zamia macrocephala*. Miquel (8) and Goepfert (6) accept Endlicher's genus, the last named adding to it three of Morris's species of *Zamites*. In 1850 Unger (16) added three more, and all seven were included by Miquel (9) under *Zamiostrobus* in his 'Prodromus Systematis Cycadearum' of 1861. In the meanwhile Corda (3, p. 84) in 1846 had suggested that the affinities of the fossil lay with the Coniferae rather than the Cycadeae, but it was left for Carruthers (2), in an important paper on Fossil Coniferous Fruits, published in 1866, definitely to establish this fact, and to show that it more closely resembled the recent genus *Pinus* than any other plant. He assigned the fossil to the genus *Pinites*, used in a comprehensive sense to include all cones of Abietinean affinity. With the aid of three other

specimens, apparently identical with *Zamia macrocephala*, L. and H., Carruthers was enabled to give a more accurate description and to correct an error regarding the geological age of the fossil. Henslow, deceived by the appearance of the sandstone in which his specimen was embedded, had referred the fossil to the 'Greensand Formation'; but Carruthers was able to fix the horizon as Eocene, for two of the three other specimens available to him were found *in situ* in a similar sandstone of this age. Of these two, one is in the Bowerbank Collection and comes from Sheppey, the other is a fossil which was found by Mr. Dowker near Canterbury, at about the junction of the Woolwich and Thanet beds. The third specimen, a water-worn fossil of unknown locality, belonging to the Cowderoy Collection, furnished the slides of *Pinites macrocephalus*, Carr., used in the following description. Since then several other specimens have come to light; Mr. Edgell's specimen of *P. macrocephalus*, in the Sedgwick Museum, was obtained from the London Clay at Reculvers, Kent.

A cone similar to *Zamia macrocephala*, L. and H., though somewhat smaller, which was found on the Kent coast near Faversham, was described by Lindley and Hutton (7, vol. iii, p. 189, Pl. 226 A) in the Fossil Flora under the name of *Zamia ovata*. Owing to its resemblance to *Zamia macrocephala* they referred it also to the Greensand. This fossil has accompanied the other in all its vicissitudes of generic terminology and was also placed by Carruthers in the genus *Pinites*. He considered it, too, to be of Tertiary age, both on account of its resemblance to *P. macrocephalus* and on account of the locality from which it was derived. The place of origin of the specimen of *P. ovata* in the Cowderoy Collection is also unknown.

Since the work of Carruthers little has been added to our knowledge of these cones. Mr. Starkie Gardner (5) in his Monograph of the British Eocene Flora in 1886 includes both cones in the recent genus *Pinus*, preferring to retain the name *Pinites* for coniferous remains from the Secondary rocks. Except that he regards *P. ovata* as only 'possibly a distinct species' he introduces practically no change into Carruthers' description.

GENERAL DESCRIPTION.

The shape of the cone can be seen from the longitudinal section of *Pityostrobus macrocephalus* (Fig. 2, Pl. XV). It is broadly ovoid-cylindrical and obtuse at both ends. This section, 9.3 cm. in length by nearly 5 cm. in breadth, has been prepared from a considerably water-worn specimen; the occurrence of part of a large seed at the very base makes it probable that the cone from which it was cut was incomplete. The cone from the Sedgwick Museum is about the same length though slightly broader, and certainly represents only the uppermost part of a much larger

specimen. Carruthers records one of the cones as being over six inches in length. *P. ovatus* is described as smaller and with a more tapering apex.

The axis of the cone is very slender, being usually less than 5 mm. in diameter, i. e. occupying hardly one-tenth of the total diameter. It is also noticeable that the axis maintains its very slender proportions almost unchanged throughout the whole length of the cone. A characteristic feature of all transverse sections is the conspicuous ring of large resin-canals surrounding the vascular cylinder.

Broad, sessile, and closely imbricated cone-scales leave the axis almost at right angles and curving sharply round the seeds continue almost vertically, though with a slight outward direction, for a distance of 4 to 5 cm. In transverse section, the cone-axis is seen to be enclosed by a whorl of four to five ovule-bearing sporophylls. Outside these is a zone, often more than 1 cm. in thickness, consisting of the erect distal portions of closely imbricated scales.

The presence of a short subtending bract-scale has been detected in both cones. In both cones also each of the large ovuliferous scales bears two ovules at its base occupying hollows in the upper surface. The distal end of the ovule is attached to the scale by a massive stalk-like tissue, while the blunt-ended micropyle is directed towards the centre of the cone. The lower part of the scale is also somewhat hollowed out underneath to make room for the ovules of the scale below; the basal region, however, near the point of attachment, is much thicker than the rest of the nearly horizontal portion. The long vertical portion remains thin until it reaches the surface, where it swells out somewhat to form the slightly projecting apophysis. The smooth and rounded surface of the latter does not appear to have been marked by any sharply outstanding umbo, but it is too worn definitely to settle this point.

The shape of the apophysis is supposed to constitute an important difference between the two so-called species. According to Carruthers, *P. macrocephalus* is distinguished by thick, flat, and irregularly six-sided apophyses, while those of *P. ovatus* are described as sub-quadrangular and higher than broad. Compared with figures of type specimens of the above species, the Sedgwick Museum cone appears somewhat intermediate in character (Fig. 1, Pl. XV). It has been referred to *P. macrocephalus*, but the apophyses are generally more quadrangular than hexagonal and are not infrequently higher than broad. Hence, even if allowance be made for the incomplete nature of the specimen, and it is noticeable that in Henslow's original drawing of *P. macrocephalus* the apophyses are markedly hexagonal even in the uppermost region of the cone, it is doubtful whether any great emphasis can be laid on the above distinction.

The breadth of the scale increases gradually from the base upwards, being about 2 cm. near the apophysis. Carruthers gives a fairly good restoration of the scale of *P. macrocephalus* (2, Pl. XXI, Fig. 6), but his figure seems to show too great a contrast between the breadth of the apophysis and the rest of the vertical portion. Carruthers also describes a striking feature which he says is peculiar to *P. macrocephalus* and *P. ovatus*, viz. that the basal scales are larger than those of the body. This is not found in any recent Abietinean cone. He says further, 'The basal scales are barren and the apophyses arise from their whole surface; in the series immediately above them there is a short flat body to the scale, but the greater portion of the scale is covered with the apophysis; the third series are fertile and have a larger and more ascending body'. Mr. Gardner in his monograph quotes and confirms this, but in the longitudinal sections which he figures, one of which is from the Cowderoy Collection, the base of the cone is too worn to show more than that the basal scales are at least very nearly as long as those higher up. The lowest scales in this section are not barren, but the upturned portion has the appearance of a long thin apophysis, so that it may be true to say that the basal apophyses are largest.

ANATOMICAL DETAILS.

1. The Cone-axis.

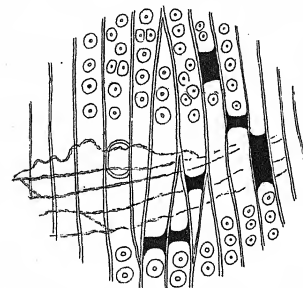
The greater part of the slender axis is occupied by a pith composed of rather thick-walled cells, more or less circular in cross-section, and often separated by conspicuous intercellular spaces. From the longitudinal section it is seen that the cells are elongated in the vertical direction, with horizontal or slightly oblique cross-walls, and, in fact, are closely similar to those in the axes of recent species. The weakly developed vascular cylinder has an external diameter of about 3 to 5 mm., but the width of the xylem ring is very small. The limits of the original bundles are usually well marked, even where there is a more or less complete ring of wood. The xylem consists of regularly arranged radial strands of tracheides separated by uniseriate medullary rays. There are no indications of centripetal xylem. At certain points in the ring narrow gaps mark the exit of the sporophyll-traces. The tracheides of the secondary xylem show abundant pitting on their radial walls. The large circular pits, which may extend over the whole length of the tracheide, are rather irregularly disposed. They are generally uniseriate and not in contact, but biseriate pitting is not rare, and in a few cases the pits are alternate and, exceptionally, with flattened margins. According to Thomson (14) this type of pitting occurs also in the cone-axes of recent species. He remarks: 'In the primitive regions of the latter (Abietineae) there is

a considerable amount of resemblance to the Araucarineae. Instead of opposite pitting, pitting in the cone-axis and early wood of the Abietineae has characteristically either scattered uniseriate pits or biseriate ones which are alternately arranged. Sometimes even the pits are flattened by mutual contact.' The pore of the bordered pit is usually circular, but sometimes elongated and oblique. In the latter case 'crossing' of the pores of superposed bordered pits on opposite walls of the tracheides was often observed. In a very few instances faint white lines were observed between the bordered pits, but for the most part no evidence of the presence of rims of Sanio could be detected.

A remarkable feature is the entire absence of resin-parenchyma or of resin-canals. This is confirmed

by the examination of transverse sections. On the other hand, what are probably resin plates or 'spools' are of common occurrence in many of the tracheides. It is interesting to notice that Thomson finds resin tracheides to be present in the cone-axes of species of *Pinus*, and he regards this as a retention of the primitive condition which is found in the Araucarineae and Cordaitales.

Trabeculae, or incomplete septations of the tracheides, occur in several places. They may, sometimes, be difficult to distinguish from the walls of the medullary ray cells, but are



TEXT-FIG. 1. *P. ovatus*. Radial longitudinal section of wood from base of cone, showing resin-spools, bordered pits, and walls of medullary ray cells. Cowderoy Collection, V 11003 e. Diagram from a photograph.

usually thicker and better preserved. The medullary rays are not well preserved. They are usually several cells in height, but always uniseriate. In almost all cases, and large numbers are to be found in the radial sections from the Sedgwick Museum cone, the course of the medullary rays is markedly oblique to that of the tracheides. Owing to the poor state of preservation and to the thickness of the sections, the details of pitting are only observable with difficulty; where clearly seen the walls exhibit some indications of 'Abietinean pitting'. No definite ray tracheides were noted, but sometimes the shape of the marginal cells of the ray is suggestive in this connexion. The tracheide field is occupied by 1-2 large 'Eiporen'; these pits are most conspicuous in transverse sections of the cone-axis. The cambium and phloem, which are not always preserved, are composed of rather crushed, thin-walled cells filled with carbonaceous

matter. The cortical region consists of thick-walled cells circular in outline. It is marked by the presence of large, regularly disposed resin-canals arranged in a circle round the vascular cylinder. The canals are very large in the case of *P. ovatus*; in the Sedgwick Museum cone they are slightly smaller and less uniform. In all the transverse sections of *P. ovatus* the canals are exactly thirteen in number. The transverse section prepared from the Sedgwick Museum cone of *P. macrocephalus* is unfortunately not complete, but about three-quarters of the axis is present and is provided with nine resin-canals, so that, in this case also, the total number was probably thirteen.

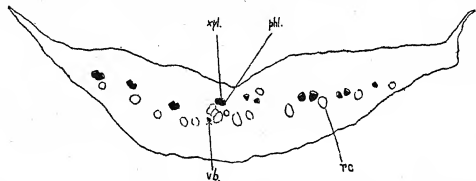
Although such a strikingly large and regular set of canals is not to be found, as far as I am aware, in any other recent or fossil Conifer, a series of canals, varying in diameter and disposition, is of frequent occurrence in the cone-axes of many existing Abietineae. Moreover, Radais (12) has shown that in recent species the number of canals may be similarly constant, and represents the number of orthostichies; for the canals run vertically between the orthostichies, giving off branches which accompany the sporophyll traces. The latter, the 'canaux appendiculaires' of Radais, can be well seen in the transverse sections of these cones (Fig. 6, Pl. XV). Since we have thus every justification for assuming that the case here is exactly as in the recent species, we may conclude that the denominator of the phyllotaxis fraction is thirteen, and this number fits in with the ordinary Fibonacci series. Henslow made a diagram of the phyllotaxis of his specimen of *P. macrocephalus*, and concluded that the arrangement was represented by the fraction $\frac{13}{5}$. Carruthers rightly points out that this is an anomalous arrangement, but from his examination of Mr. Dowler's specimen of *P. macrocephalus* he determined the ratio as $\frac{8}{3}$. From the considerations given above it is probable that the real value in both cones must be the next in the series, viz. $\frac{5}{2}$.

2. The Bract Scales.

Neither Carruthers nor any of the later writers makes any mention of the bract-scales. Yet evidence on this point is not lacking. In longitudinal sections of *P. macrocephalus* the bract-scale could sometimes be identified as a stout, pointed body inserted below the ovuliferous scale and occupying the space just above the micropyle of the ovule below. A similar structure was noted in a longitudinal section from the base of the cone of *P. ovatus*. A transverse section of such a scale can be seen in the tangential section from the same cone (see Fig. 4, Pl. XV). This is composed of compact, thick-walled tissue and possesses a row of small resin canals but no vascular bundle. In no case has it been possible to make any observations on the vascular supply of the bract-scale.

3. *The Ovuliferous Scales.*

The tissue of an ovuliferous scale consists chiefly of rather large, very thick-walled cells, often showing contents. In the surface layers the cells are smaller and more strongly sclerized. It is noticeable, however, that the scale as a whole, and particularly the basal region, as seen in transverse section, does not show such a large development of sclerotic tissue as seems to be usual in recent cones of a similar size. The difference in the thickening layers of the upper and lower surface is most clearly exhibited in the sections from the Sedgwick Museum cone. The lower layers of the scale consist of very narrow elongated sclereids appearing as fine wavy lines in a longitudinal section, and as crowded, circular areas with minute lumina in a transverse section of the scale. The layers of the upper surface consist of larger sclereids with polygonal outlines and frequently contain massive cells



TEXT-FIG. 2. *P. ovatus*. Transverse section of ovuliferous scale about half-way up, showing disposition and orientation of vascular bundles and chief resin-canals. *vb.*, vascular bundle; *xyl.*, xylem; *phl.*, phloem; *rc.*, resin-canal. Camera lucida drawing. $\times 6$. Cowderoy Collection, V 8309.

with peculiar irregularly elongated shapes, which may be compared to the idioblasts found in the cone-scales of the Araucarineae, &c. In the larger middle region the cells are only moderately thickened, and the whole tissue is permeated with a profusion of secretory canals. In *P. ovatus* the upper sclerized region is not so large, nor so clearly delimited from the middle region.

The seed-scale is supplied by two separate vascular strands arising independently from the sides of the gap in the main stele, just as Miss Aase (1) has recently shown to be the case among many recent conifers. Most transverse sections of the cone show the two bundles very clearly (Fig. 6, Pl. XV). The scale traces quickly broaden out and divide so as to form a single series of bundles with phloem uppermost. Text-fig. 2 shows the distribution of the bundles in a cross-section about half-way up the vertical part of the scale. The small size of the bundles and their frequent arrangement in pairs are noticeable features. In *P. macrocephalus* the bundles, though more numerous, seem to be even more minute than represented in

this figure. Some of the bundles have been cut obliquely longitudinally, and such sections show the tracheides of the xylem with uniseriate bordered pits on the walls appearing as oval black 'discs'. Protoxylem elements with spiral thickenings may be found at the edge farthest from the phloem. The bundles in the middle region of the scale are often inclined to one another at various angles, and in some cases are so grouped as to suggest the incipient formation of a second series: this is particularly the case in Mr. Edgell's specimen of *P. macrocephalus*. Dr. Stopes (13) records a similar condition in the case of *Pinostrobus sussexiensis*, Mantell, from the Lower Greensand, but in this cone the irregular orientation is more marked and a second series of bundles is definitely present,

4. The Ovules.

These form one of the chief features of interest. In a number of cases the contents of the ovules are comparatively well preserved and the relationships of the different structures can be made out with great clearness. The integument is thick and tapers at the apex to form a long blunt-ended micropylar tube, usually nearly or quite closed, and which is strikingly similar in appearance to that of certain Cycadean types. A transverse section in this region shows that it is composed almost entirely of heavily lignified tissue and has a narrow slit-like micropylar opening (see Fig. 4, Pl. XV). This, of course, is common in recent Gymnosperm ovules. Some of the ovules are flattened in the plane of the scale on which they lie, and in some seeds of *P. ovatus* the lateral edges are so distinctly ridged as to give a marked impression of bilateral symmetry. It is probable that the ridges are partly caused by the pincer-like extensions of the wings along the edges of the seeds from the base upwards. In the large transverse section of an ovule at the left of Fig. 4, Pl. XV, a crack appears to penetrate each of the lateral ridges, but, as a matter of fact, the opening is closed to the exterior. In the large ovule on the right-hand side of the same photograph the passage seen is actually complete, although the section seems to be quite transverse and much below the micropylar opening. The effect is probably due to external pressure. The base of the ovule frequently exhibits an extensive wing tissue at its point of attachment (see *W.*, Fig. 3, Pl. XV). Comparison may be made with Tubeuf's (15) figure of a longitudinal section of an ovule of *Pinus excelsa* which shows the large parenchyma developed by the wing in the same region. There seems no ground for Mr. Gardner's (5) statement that in *P. macrocephalus* the seeds are possibly wingless.

(i) The Integument.

The thick wall of the integument shows a differentiation into at least three layers. The structure, which can be studied to the best advantage in the well-preserved ovules of the Sedgwick Museum cone, is identical in all

three specimens. There is an outer layer which has usually been completely carbonized, though in some places the crushed remains of thin-walled cells adhere to the inner layers. From the manner of preservation it is clear that this represents the remains of an outer fleshy layer. Next comes a wide zone of well-preserved, thick-walled cells, obviously representing a 'stony layer'. As the photographs show, the outline of this towards the exterior is very irregular, indicating that the surface must have been marked by projecting ridges, which in some places are very prominent. The testa of the ovule of *Pinoostrobus sussexiensis*, already referred to, appears to have possessed much the same structure. In reference to this Dr. Stopes remarks, 'In section, the stony layer of the testa is much indented and is irregularly star-shaped in outline, which appears to represent surface-corrugations. How far these are natural, and how far petrifact, the sections do not afford data to determine' (18, p. 127). In the less well preserved tissue of the integument of *P. ovatus* the interior of this stony layer has often been partly decomposed, leaving spaces filled with black, carbonaceous granules. This is especially common in the interior of the larger ridges.

Towards the inside the stony layer merges gradually into a less heavily sclerized zone, the cells of which are rendered conspicuous by their polygonal outlines and distinct lumen. These cells are more or less isodiametric, while those external to them are usually somewhat radially elongated. On the inside again there is a transition to small thick-walled cells, but their structure is not easily discernible and they appear to pass insensibly into the layer of crushed, fibrous-like cells which form the inner lining of the integument, and which has often become separated from the main part of the wall. This is very noticeable in the ovule seen in Fig. 9, Pl. XV, where the inner layer is only in connexion with the ovule at the very base.

(ii) *The Nucellus.*

Except in the apical region, only a thin layer of this tissue has been preserved. In the ovule mentioned above (Fig. 9, Pl. XV) it might appear at first sight as if the nucellus were free from the integument from the base upwards, but on a little closer examination it becomes evident that the nucellus is coalescent with the separated wall of the integument and only becomes free in the upper portion. In this ovule, moreover, the relations of the different structures are rendered perfectly clear by reason of the separate, distinct outline of the small crumpled prothallus which occupies the centre. Many of the other sections, however, are more difficult to interpret. A good example is the well-preserved ovule of Fig. 1, Pl. XV. In this ovule it would seem as if the cavity were nearly filled by a well-rounded prothallus which exhibits an extraordinary beak-like prolongation in its apical region. The rather shadowy surrounding tissue appears to be lifted up by the beak, so that the whole structure at once calls to mind the

way in which the 'tent-pole' of *Ginkgo* lifts up a cap formed by the remnant of the nucellus. The analogy, however, turns out to be quite false because from closer examination and comparison with other ovules it can be made out that the apical column or beak is composed of nucellar tissue, while the 'cap' forms part of the separated inner layer of the integument. In the first place it is not difficult to recognize that the thick layer surrounding the prothallus consists of much more than the megaspore membrane alone. Presumably the latter does line the interior, since the whole structure is filled with remains of prothallial tissue, but it is fairly certain that the nucellar membrane is responsible for much of the thickness. This view derives much support from the fact that at both shoulders of the large prothallial body, part of the membrane is seen to become fused with the inner integumentary tissue. Moreover, where the structure of this membrane can be determined, as, for instance, in the apical region, it is found to share all the characteristics of the nucellar tissue of other ovules. We may conclude, therefore, that we have to deal with an ovule, at a fairly advanced stage of development, in which the prothallial tissue has become much enlarged and the nucellus reduced to a mere lining layer, only part of the original prolongation being still preserved. The nature of this prolongation is discussed later on. Even in this ovule the nucellus has not altogether shrunk away from the integument. It is the inner layers of the latter which have separated from the main wall of the ovule, but yet adhere in several places to the nucellar tissue; thus also taking some part in the formation of the composite lining of the prothallus.

Turning now to other ovules for further verification, it may be noticed first that very similar conditions are to be found in one of the ovules directly below that just described. Here in one place, part of the rather delicate megaspore membrane has become separated from the thick surrounding layer, leaving no doubt that the latter is mainly of nucellar origin. In the other ovules shown in this longitudinal section of the cone, the prothallus, and consequently also the nucellus, has rarely such a well-rounded form as in two examples just cited; both nucellus and prothallus are usually much shrunken. On the other hand, in another longitudinal section which is taken from the same cone, there are some well-preserved ovules in which the nucellus is in contact with the integument for the greater part of its length (see Figs. 11, 12, Pl. XV). The nucellus lines the integument wall right up to the apical region, where it becomes free and extends across the cavity of the ovule. In both cases there is a large megaspore with a clearly defined membrane. In the ovule of Fig. 11, Pl. XV, the inner layer of the integument has separated somewhat at the apex of the ovule.

It is a significant fact that in the sections of *P. ovatus* those ovules which have been cut at all longitudinally exhibit very much the same

sort of internal structure as those of *P. macrocephalus*. The nucellus partly lines the integument and is partly free from it. It is always in connexion with the integument at the shoulders of the ovule, and there are nearly always indications of a well-developed apical column. Fig. 10, Pl. XV, which represents the upper part of a large ovule cut obliquely longitudinally, gives some idea of the stage of development shown in these sections. In this, as in many other examples, the structural features of the nucellar tissue are well preserved. They can easily be made out in the patch of tissue on the right-hand side of the photograph, especially when examined with a lens. The tissue is rarely more than one cell layer in thickness, and the thin walls of the separate cells have often been finely preserved. The cells are comparatively uniform in size, the whole structure forming a network of narrow elongated meshes. The characters just mentioned afford a criterion by means of which it is possible to recognize the nucellus in several cases which would otherwise be doubtful. Thus it often happens that by this means it is possible to distinguish both nucellar and integumentary tissue in a layer in which the double morphological origin is not immediately obvious. It, of course, affords decisive evidence of the nucellar nature of the tissue surrounding the prothallus in the ovule of Fig. 9, Pl. XV.

(iii) *The Nucellar Column.*

More or less definite evidence of the existence of this peculiar feature is to be found in almost every longitudinal section of an ovule. It is evident from the photographs (Figs. 7, 10, 11, 13) that this structure does not form a nucellar beak, but is rather a cylindrical column of parenchymatous or somewhat thick-walled cells which may exhibit the typical structure of the nucellar tissue as seen elsewhere. This parenchymatous structure is especially distinct at the base of the column where there is a shallow, moat-like depression (see Fig. 13, Pl. XV).

In the ovules of the Sedgwick Museum cone the structure of the nucellar apex is exhibited with exceptional clearness. The typical columnar form is usually represented, but the darker opaque portion appears irregularly broken into bands separated by clearer spaces, and is very suggestive of permeation by a number of pollen-tubes (see Fig. 15, Pl. XV). The cellular framework is also coarser than usual—the cells being elongated and the walls thicker and darker, though the characteristic fine meshwork tissue is often evident in the lower part of the column. There is one case in which only the thick outer wall of the column has been preserved, and this is not continuous across the apex, so that here the beak actually appears as a hollow open chamber with contorted sides.

In the thicker sections from the other cones where the whole of the beak is seen it almost invariably is dark-coloured and opaque or encloses an opaque

mass of substance, which makes it practically impossible to determine the nature of the tissue. The evidence is in favour of the view that the basal part, at least, is hollow. In the ovule seen in Fig. 10, Pl. XV, the opaque body appears to extend right into the tissue of the prothallus, but even here the bounding layer of the megaspore is found to be continuous round the intrusion. In some cases where the longitudinal section of the ovule is rather out of the median, the beak is not represented at all, but its position is indicated by a gap in the nucellar tissue which stretches across the apical region; the edges of the gap being somewhat upturned. This appearance (see *M.*, Fig. 3, Pl. XV) lends support to the view that the base of the beak was originally hollow. In one of the ovules from a small tangential longitudinal section there occurs a somewhat similar, but much narrower gap in the upper part of the nucellus, which is peculiar in being continued below through the megaspore-membrane as a sharply curved channel in the tissue of the prothallus. At the margins of the channel can be seen elongated cells somewhat like those of the nucellus, but larger and with more resemblance to the cells of the endosperm tissue, which, as a matter of fact, can only be made out in this particular slide. It is possible that the cavity has been formed by the invasion of the prothallus by a pollen-tube, although no trace of the pollen-grain can be found. It must, however, be remembered that the section is very oblique.

(iv) *The Female Gametophyte and Embryo.*

All the ovules seen in the larger sections contain prothallial tissue at about the same stage of development, but the actual state of preservation is somewhat different in different sections. An unusual condition is to be found in an ovule from the base of the cone of *P. ovatus* (Fig. 10, Pl. XV), the centre of the ovule being occupied by a small contracted megaspore with a much crumpled outline. In the majority of cases the prothallus is fairly large, though its membrane has often been somewhat contracted. Very often, especially in the Sedgwick Museum cone, there seems to have been little or no shrinkage, the outline being well rounded and closely surrounded by the nucellus (e. g. Fig. 9, Pl. XV).

The megaspore membrane consists of a comparatively thin brownish layer. Traces of prothallial tissue can nearly always be found within the megaspore; as a rule the individual cells are not distinguishable, though there is a close network of faint yellow lines and granules which is clear enough evidence of the nature of the tissue. The individual cell-walls could only be distinguished in one of the tangential sections where the ovule had been cut across transversely, and where at one side the prothallial tissue had contracted from the megaspore wall. In this region the outer part of the prothallus is seen to be composed of very thin-walled, more or less isodiametric, polygonal cells.

Very often the prothallus contains a large central cavity, or sometimes there may be more than one. In the Cowderoy specimen of *P. macrocephalus* these clear spaces are usually surrounded by an opaque black substance, largely of carbonaceous nature, which has probably been derived by the decay of the prothallial tissue that has shrunk away from the centre of the cavity.

The tissue of the gametophyte is remarkably homogeneous. With two exceptions not only are there no signs of an embryo, but there is not even evidence that, at the time of fossilization, differentiation of archegonia had taken place, even in the largest ovules. The two exceptional ovules were found in the cone of *P. macrocephalus* belonging to the Sedgwick Museum. In both the prothallus contains a body which undoubtedly represents a nearly mature embryo (Figs. 11, 12, Pl. XV). The better preserved of these is of typical cylindrical shape, tapering at the ends and contracted below the insertion of the cotyledons, and is surrounded by a tapetum-like jacket of endosperm cells which can be exactly matched in recent pine-seeds. The cells of the hypocotyl, though not individually preserved, have every appearance of being arranged in regular meristematic rows, but the cotyledonary apex is rather decomposed, so that neither the growing point nor the separate cotyledonary lobes can be distinguished. In this embryo the extremity of the radicle is very close to the base of the nucellar column, and it is interesting to notice that at the apex of the latter there are a number of structures which can only be interpreted as germinated pollen-grains (Fig. 15, Pl. XV). A suspensor could not be demonstrated; but in view of the age of the embryo this is not surprising.

In the other case the embryo exhibits at its apex a whorl of cotyledonary lobes with indications of a growing-point at their base; but otherwise it is less well preserved, and the middle region has become doubled up, presumably owing to the contraction of the apical region of the prothallus (Fig. 12, Pl. XV). No trace of pollen-grains or of pollen-tubes could be found in this ovule.

(v) *The Male Gametophyte.*

At the apex of the nucellar column of the ovule shown in Fig. 7, Pl. XV, there are two small rounded objects which most certainly represent two winged pollen-grains; they can be seen more clearly under the higher magnification of Figs. 13, 14, Pl. XV. The wing exhibits the characteristic reticulation seen in the pollen-grains of living pines, but this feature does not appear clearly in the photographs. These pollen-grains show no signs of germination.

On the other hand, it has already been remarked that structures which must be considered as germinated pollen-grains occur at the apex of the nucellar column of one of the fertile seeds. These form a small

collection of three or more grains, but the outlines are so indistinct that it is hardly possible to say whether a wing is present or not (see Fig. 15, Pl. XV).

No other examples have been found in any other ovule. Some much smaller spherical bodies, with a thin wall and filled with contents, have been observed in the neighbourhood of the nucellar apex; these seem to represent some sort of fungal spore. The nucellar apex often appears to have been perforated by pollen-tubes (this is evident in Fig. 15, Pl. XV), and a possible example of the invasion of the prothallus by a pollen-tube has been mentioned already.

THEORETICAL CONSIDERATIONS.

Before proceeding to discuss the significance of the special features associated with the cones just described, it will be as well to consider briefly the grounds on which it may be inferred that we are dealing with a single species only. The two species to which all the present material has been referred were founded entirely on differences in external appearances. Carruthers mentions only differences with respect to the form of the cone and the shape of the apophyses. His diagnoses read:

1. *Pinites macrocephalus*. Cone cylindrical, obtuse at both ends; scales with thick and flat, irregularly six-sided apophyses; basal scales largest.

2. *Pinites ovatus*. Cone ovate, with a truncate base and obtuse apex; scales with thickened, flat, sub-quadrangular apophyses; basal scales largest.

He states in his description of *Pinites ovatus* that this cone can be readily distinguished from that of *P. macrocephalus* 'by the form of the apophyses of the scales, which are longer than they are broad, and quadrangular or sub-quadrangular, the upper and lower angles being acute or but slightly truncate'. As to the general validity of this I am unable to judge, but some doubt has already been thrown on the value of this criterion in view of the intermediate character of the apophyses of the Sedgwick Museum cone.

A conspicuous difference in the appearance of the slides examined is to be found in the darker colour of those of *P. macrocephalus*. This is particularly noticeable in the Cowderoy specimens. It is partly due to the greater thickness of the sections, but largely also to their siliceous matrix being actually darker and containing a larger proportion of black carbonaceous matter. In all cases, however, the actual tissues preserved are exceedingly alike in form, structure, and arrangement. In both forms the scales possess the same general shape, with a nearly horizontal proximal portion, and, according to Carruthers, both possess the long basal scales which form so distinctive a feature of these cones. Another characteristic

feature shared by both forms is the very slender cone-axis with its surrounding ring of uniform resin-canals. As far as can be judged the number of these canals does not vary in the different forms, and the consequent identity in phyllotaxis ratio constitutes another point of agreement between the cones.

With regard to the ovules, the similarities are equally well marked. The integument, the nucellus, and the prothallus are conspicuously alike in structure and in state of preservation. Mr. Gardner's view that in *P. ovata* 'the seeds seem rounder' is not borne out by any of the slides examined. It is especially noticeable that the characteristic nucellar column and the well-developed gametophytic tissue are to be found in all the preparations. On the whole it seems justifiable to state that there are no important anatomical differences between the two so-called species. Taking this into account it seems probable that the differences in external character will turn out to be of less than specific value. At any rate it will be seen from what has been said that we can proceed with our discussion as if we were dealing with a single uniform type.

We may now turn to the consideration of some features of the cone which seem to distinguish it from existing species of *Pinus*. In the first place must be mentioned the very long and often fertile basal scales. Assuming that this is a real feature and not due to the base of the cone being broken off, it would seem that Carruthers is quite right in saying that it is a structure peculiar to these cones but not sufficient to separate them from the fossil genus *Pinites*. The very slender cone-axis is a character of some importance. This is accompanied by a very weak development of the vascular tissue as a whole, which thus does not appear to show any correspondence with the demands which must be made upon it by the reproductive organs. The wood of the very narrow xylem cylinder, often separated into distinct bundles, is always of a very homogeneous character, the simple uniseriate medullary rays and the absence of resin-canals being particularly noticeable. If we regard the cone-axis as a seat of ancestral characters, it would seem that the presence of resin tracheides in the place of the usual ducts must be considered as a primitive feature. It is in accordance with the general weak development of the conducting tissue as a whole that we find that the sporophyll-traces are very small, and that the scale strands to which they give rise are also small and comparatively widely separated even in the basal region. In the nearly ripe cones of recent species of *Pinus* that I have examined the fairly thick vascular cylinder becomes markedly angular in the region of insertion of the large scale supply, and the latter divides up almost immediately to give a row of bundles nearly touching each other and with a considerable development of secondary xylem. Although, however, it has not been found possible to match in the recent species the extreme reduction of the vascular tissue that

we find in this case, there is no doubt that this feature is of no more generic value than the fact that the resin-canals surrounding the axis are more uniform in size and more regularly distributed than appears to be the case among the present-day representatives.

The same thing really applies to the peculiar ovular characters, however difficult their interpretation.

The large size of the ovules, the detached seed-wing, the lignified integument, with closed or almost closed micropyle, and the shrunken nucellar layer, all seem to favour the view that the cone is well on its way to maturity. This is borne out by the appearance of well-developed embryos in two of the ovules. On the other hand, there is generally no indication of either archegonium or embryo, in spite of the large prothallus, and prima facie it would be natural to conclude that the cone is really much more unripe than its size and general structure would appear to indicate. The presence of ungerminated pollen-grains would appear to support this conclusion and suggests the inference that the cone may have become detached from the tree very shortly after pollination, and its development checked before fertilization was possible. On the other hand, in view of the comparatively advanced stage of development of the two fertile seeds, the prevalent absence of both pollination and fertilization can hardly be ascribed to immaturity.

The ovules are rendered still more striking by the presence of the peculiar nucellar beak. This is a cylindrical structure and does not appear to have been open at the top, so we have no grounds for supposing that it could possibly have functioned as a pollen-chamber. This is confirmed by the peculiar position of the pollen-grains. From examination of the old slides I was inclined to regard the columnar appearance as having resulted from the shrinkage of what was originally a massive apical region to the nucellus. A parallel might then be drawn with the massive free upper part of the nucellus that Dr. Stopes records as occurring in an immature ovule of *Pinostrobus Benstedii* from the Lower Greensand. There, however, the nucellar apex is conical in shape, and the attenuated tip engages with the base of the micropyle. The ovule, also, is so young that the comparison is of doubtful value. Moreover, the appearance as seen in the new slides of *P. macrocephalus* is so definite and constant that it is difficult not to conclude that the structure is a normal and characteristic feature of these ovules.

The presence of the beak, coupled with the general absence of any embryonal structures, suggests a comparison with Pteridosperms, which, however, is perhaps more interesting than profitable. Oliver and Scott (10 a), speaking of *Lagenostoma Lomaxi*, say, 'The seed of *Lyginodendron* differed from recent seeds in the early maturing of its tissues. Already before fertilization has taken place they appear to have reached the limits of

their development and would appear to be incapable of further stretching.' It is just possible that something of the same sort may have occurred in these cones.

Oliver and Scott (10 a) also speak of 'the well-preserved tissues of the nucellar base'. In our ovule the nucellus is reduced to a thin layer, while the endosperm seems more developed than in *Lagenostoma*. If then the absence of an embryo is striking in the latter instance, it should be quite as much or more so in the case of *P. macrocephalus*.

Coming finally to the consideration of the affinities of the present specimen, it will hardly be necessary after what has been said to enumerate the features which make it more closely allied to the modern *Pinus* than to any other genus of the Abietineae. The general shape and size of the cone, the two sorts of scales and their relative importance, the two reversed ovules on each scale, the winged seed, all make it certain that the cone is in every essential character identical with the living genus. Special peculiarities there are, but we have already seen that none of these can be considered to be of generic value.

It is not at all so easy to make sure of its relations within the genus *Pinus* itself. Carruthers instituted a comparison of the internal structure with that of *Pinus Pinaster*, while from the apophyses he concluded that the affinities were with *Pinus pinea*. Of the latter feature he says, '(The apophyses) scarcely differ from those of *Pinus pinea*. . . . Indeed in the form, size and arrangement of the apophyses, this recent pine remarkably resembles the fossil cone.' But, as Carruthers admits, the form of the cone and the internal structure are very different, and it seems to me that there can be no comparison between the two cones. Nor has our cone much in common with *Pinus Pinaster*. There is some general resemblance in the form of the scales, but the pyramidal shape of the cone, the very stout axis, and the small pointed apophyses make the recent species a quite different type. It is unfortunate that information is lacking as to the crucial feature of the position of the umbo on the apophysis. Carruthers would have us believe that the apophysis was a large flat surface with possibly a small central umbo. At any rate, all his suggestions of affinity refer to the *Pinaster* section of the genus. But, as already pointed out, one cannot place a great deal of reliance on his restorations, and it seems more probable that the umbo was terminal on the apophysis, as in the section *Strobus*. The general features of the cone, such as the very slender axis, are in accordance with this view of its affinities. It is interesting to note also that there appear to be many resemblances between this cone and that of *Pinostrobus sussexiensis*, Mantell., from the Lower Greensand, which is generally agreed to be closely related to *Pinus Strobus*, L. The pitting of the medullary ray field agrees with that in the recent *Strobus* section, and denticulate ray tracheides are, at least apparently,

absent. It may be noted, also, that Radais, speaking of the anatomical distinctions between the two sections of *Pinus*, remarks the absence of resin-canals from the vascular system of the scales, and apparently also from the wood of the axis in the section *Strobis* (12, p. 278). Resin-canals are, however, present in the wood of the cone-axis of the species *Pinus excelsa*. On the whole, the anatomical characters of *P. macrocephalus* agree with those of species belonging to the *Strobis* section, and, while there is no very close agreement with any of them, the fossil seems to have more in common with *Pinus excelsa*, L., than with any of the others.

The following diagnosis is based on the cones here considered :

Pityostrobus macrocephalus, L. and H. Cone ovoid-cylindrical, apex obtuse. Length 15 cm. or less. Breadth 5 cm. Axis very slender, surrounded by a conspicuous ring of resin-canals. Scales leave axis almost horizontally and then ascend sharply beyond the seeds, which are borne in pairs in hollows on the upper surface near the base. Apophyses thick, flat, quadrangular or irregularly hexagonal. Basal scales with large apophyses. Short bract-scale. Ovules, two on each scale, 1 cm. long by 0.6 cm. in breadth. Stone layer corrugated. Seeds usually barren.

SUMMARY.

The paper gives a detailed description and comparison of the internal anatomy of two forms of cone occurring in the Lower Eocene of the London Basin.

Two separate species, *Pinites macrocephalus*, Carr., and *Pinites ovatus*, Carr., have been founded on the slight differences in external appearance, but the close resemblance in internal structure justifies the conclusion that they are specifically identical.

The new aggregate species has been called *Pityostrobus macrocephalus*, L. and H.

The cone exhibits several interesting anatomical features. The cone-axis is very slender and has a weakly-developed xylem cylinder which contains neither resin-canals nor resin-parenchyma, but resin 'spools' are present in ordinary tracheides. A conspicuous circle of resin-canals surrounds the axis, and from their number an accurate determination of the phyllotaxis of the cone can be deduced.

The cone-scales are of the normal *Pinus* type, a short bract-scale being present. The cone is, however, peculiar in the large size of the lowermost seed-scales.

The most important observations have reference to ovular structures. The ovules are provided with a thick, differentiated integument with a long, almost closed micropyle. They represent nearly mature, winged seeds, but are usually barren and with the contents little shrunken.

The nucellus is marked by a peculiar apical column of tissue. Winged

pollen-grains, both germinated and ungerminated, have been found collected at the tip on the external surface.

The prothallus is large and well rounded, but from only two of the ovules were fossil embryos recorded. These were large and, apparently, of a normal polycotyledonous type.

It is concluded that the cone has closer affinities with *Pinus excelsa*, L., than with any other existing Conifer.

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EXPLANATION OF PLATE XV.

Illustrating Mr. C. P. Dutt's paper on *Pityostrobus macrocephalus*.

The specific names are those attached to the original specimens.

Fig. 1. *P. macrocephalus*. External appearance of cone in the Sedgwick Museum, Cambridge. About nat. size.

Fig. 2. *P. macrocephalus*. Nearly median longitudinal section of cone in the Cowderoy Collection. Basal scales with large apophyses. A, Ovule seen in Fig. 7, V 11001 b. About nat. size.

Fig. 3. *P. macrocephalus*. Median longitudinal section of same cone as in Fig. 2. B, Ovule seen in Fig. 8. W, base of seed wing; M, megaspore. Cowderoy Collection, V 11001 c. x about 1½.

Fig. 4. *P. ovatus*. Tangential longitudinal section near base of cone. Ovuliferous scales bearing paired ovules; b.s., bract-scale. Cowderoy Collection, V 11003 d.

Fig. 5. *P. ovalus*. Transverse section of cone. Cowderoy Collection, V 8309. Nat. size.

Fig. 6. *P. ovalus*. Part of transverse section (Fig. 5) showing double nature of scale-trace supply; *r.c.*, resin-canals; *app.r.c.*, appendicular resin-canals accompanying the scale-traces. Cowderoy Collection, V 8309.

Fig. 7. *P. macrocephalus*. Ovule A, Fig. 2, more highly magnified; *n.c.*, nucellar column. Cowderoy Collection, V 11001 b.

Fig. 8. *P. macrocephalus*. Upper part of ovule B, Fig. 3. Not quite median section; *Int.*, part of integument separated from main wall; *Nuc.*, nucellus; *m.m.*, megaspore membrane; *End.*, endosperm tissue; *c.c.*, central cavity.

Fig. 9. *P. ovalus*. Longitudinal section of an ovule from the base of the cone, not quite median; *N.L.*, double layer of nucellus and integument tissue shrunk from wall; *Nuc.*, nucellar tissue alone, with indication of beak; *Mg.*, small contracted megaspore. Cowderoy Collection, V 11001 a.

Fig. 10. *P. ovalus*. Upper part of ovule showing peculiar nucellar apex; *n.c.*, nucellar column which appears to penetrate endosperm; *Nuc.*, nucellar tissue showing cellular structure clearly; *m.m.*, megaspore membrane. Cowderoy Collection, V 11003 c.

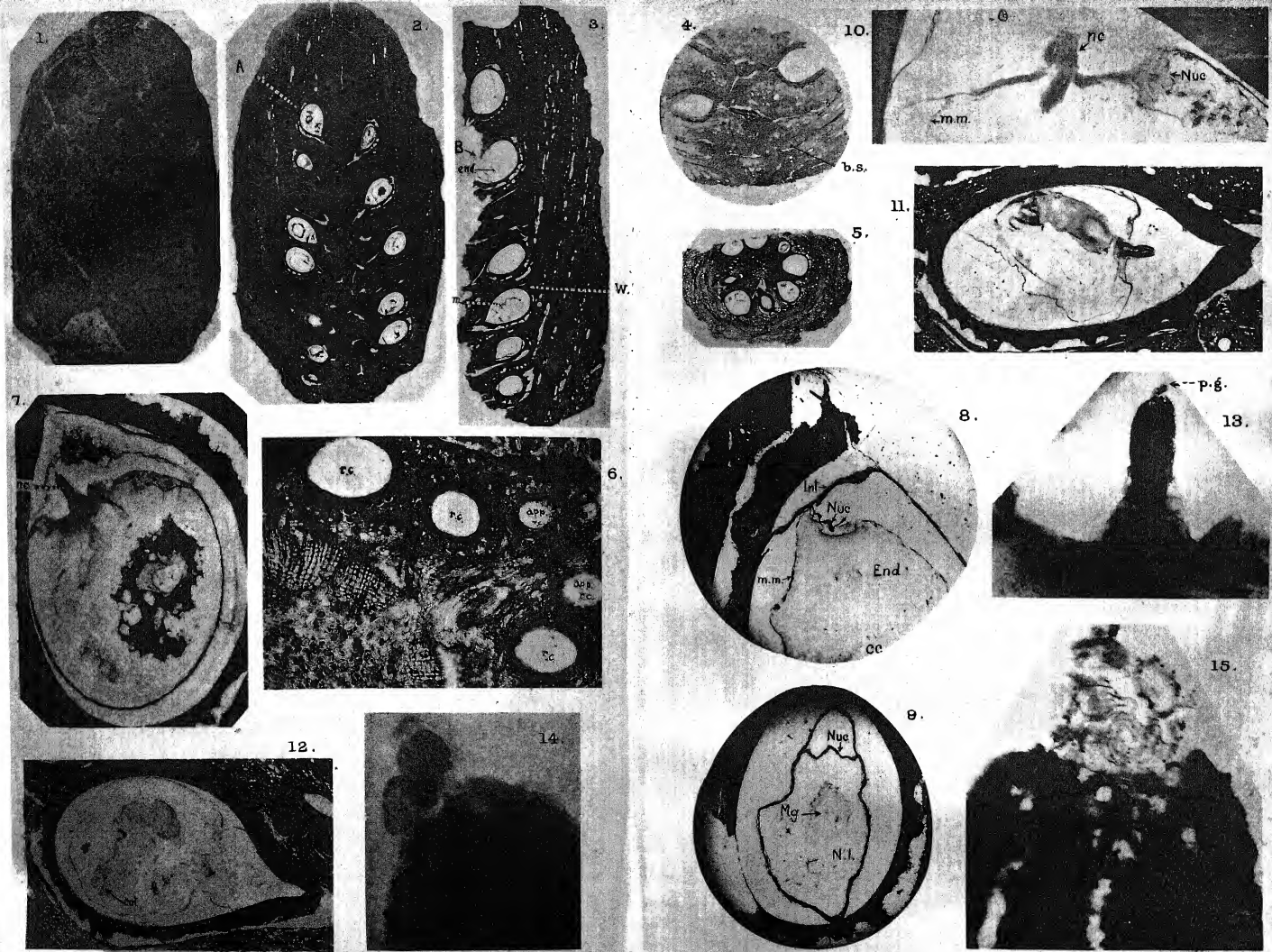
Fig. 11. *P. macrocephalus*. Longitudinal section of ovule showing large embryo and nucellar column. Sedgwick Museum.

Fig. 12. *P. macrocephalus*. Another ovule from the same cone also showing an embryo. A whorl of cotyledons (*cot.*) is present with a growing point at the base.

Fig. 13. *P. macrocephalus*. Nucellar column of ovule seen in Fig. 7, more highly magnified; *pg.*, pollen-grains. Cowderoy Collection.

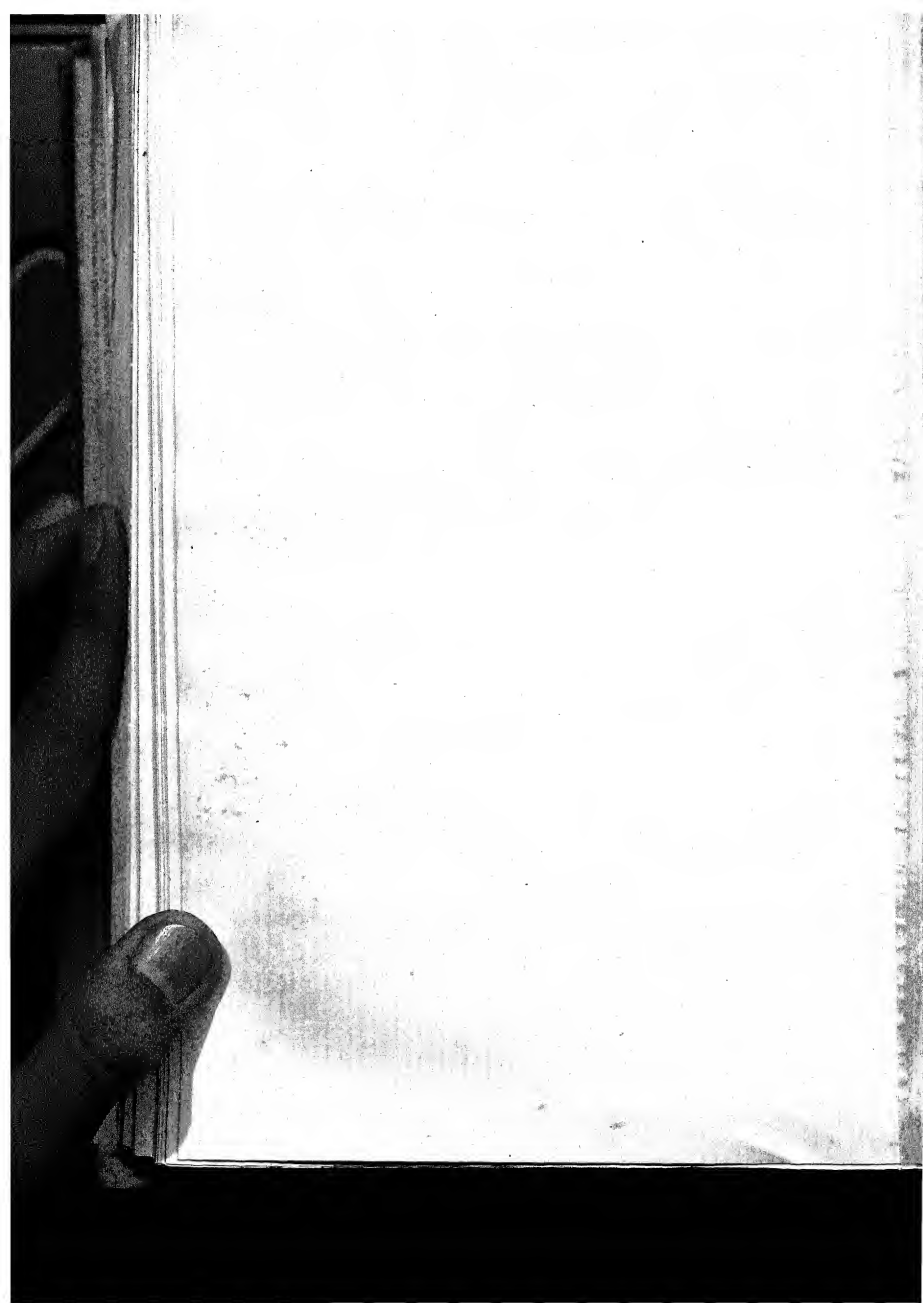
Fig. 14. *P. macrocephalus*. Tip of same nucellar column still more highly magnified, showing two winged pollen-grains.

Fig. 15. *P. macrocephalus*. Tip of nucellar column of ovule seen in Fig. 11, showing germinated pollen-grains and nucellar tissue perforated by pollen-tubes.



C.F. Dutt, et W. Tam, 1908.

DUTT - PITYOSTROBUS MACROCEPHALUS.



22. JAN 1917

On Endemism and the Mutation Theory.

BY

H. N. RIDLEY, F.R.S.

THE history of the rise and fall of species of plants or animals is a subject of great interest, and it is one which has very seldom been worked out. Dr. Willis has, however, lately, in a paper to the Royal Society, *Phil. Trans.*, B, ccvi, p. 307, and one published in the 'Annals of Botany' (vol. xxx, 1916, p. 1), attempted to formulate a law dealing with the rarity or commonness of species and its bearing on the endemic plants of Ceylon. He bases his argument on a study of Trimen's 'Flora of Ceylon', in which work Dr. Trimen states under the description of each plant whether it is very common (VC), common (C), rare (R), and very rare (VR). Now it is known that Trimen in putting these notes merely referred to the number of specimens in the Ceylon herbarium, and Dr. Willis (*Ann. Bot.*, l. c., p. 4) admits that the figures 'are based on herbarium specimens'. Dr. Trimen himself in vol. i, p. ix, writes as to general distribution and comparative frequency: 'Very much has yet to be done in tracing out the distribution of plants through the island, and the information here given is very imperfect and will be much modified and increased by further investigation.'

No further investigation on these lines has apparently been done in Ceylon. In Dr. Trimen's day Ceylon was not so easy of exploration as it is now, and even now large areas are difficult to get at, and would require a large staff of botanical collectors to take a thorough census of the various species throughout the island. I do not think that this has been done indeed for any region of the world except the British Isles.

The number of specimens of a species in a herbarium does not show at all the abundance or rarity of any given plant. Frequently a tree in the tropics does not flower for very many years, and collectors do not collect specimens which are not in flower or fruit. One of the commonest trees in Sarawak is *Koompassia parvifolia*, the Tualang. The only flowering specimen known is one in the Florence herbarium picked up by Beccari, and the only fruiting ones were collected by me last year. Thus in herbaria this would figure as VR (very rare), whereas it should be VC (very common).

Conversely, a plant only known from a single tree often in flower and easy of access might be extensively represented in herbaria, as every

collector who happened to be in its neighbourhood would naturally collect specimens.

In an extensive tropical area like that of Ceylon, to make a census of scientific value upon which one could safely base deductions, a very large staff of collectors and botanists would be required; in fact, a botanical survey such as has been of late years commenced for India would have to be organized. The number of botanists mentioned in Dr. Trimen's *Flora* is under twenty, and this includes several who only resided a year or two in the island, and practically only Hermann, Koenig, Champion, Gardner, Thwaites, and Trimen collected on anything like a large scale. Dr. Trimen did the best he could with what time and materials he had, but, as he says, his work is incomplete.

Dr. Willis (*Ann. Bot.*, l. c., p. 22) states that 'A very small accident may kill out a species while at or below the stage represented in the Ceylon classification by VR, whilst it will need a geological submergence or some such accident to kill out one represented by VC', and 'There is no evidence whatever that any of the angiospermous species of the Ceylon flora are dying out, and from analogy we may imagine this to be generally true'.

I first visited Ceylon in the autumn of 1888 and stayed for a month at Peradeniya with Dr. Trimen. At that time *Hedychium coronarium*, L., and *H. flavescens* were conspicuously abundant all round Peradeniya and Kandy, and Dr. Trimen marks both of them VC in the *Flora*, vol. iv, p. 245.

I visited Ceylon several times later in 1912 and 1913, and went to Peradeniya on these occasions; but it was not till my last visit that I noticed that both species had entirely disappeared, and I asked Mr. Lyne, the Director of the Gardens, if he had seen them anywhere, and he was surprised to hear that they had ever been common, as he had not seen a plant at all in Ceylon.

Here is a case in which a plant, which in 1888 was very common, in twenty-five years has become at least very rare, without any geological cataclysm to account for it. It is difficult to give any reason for its disappearance, as no observer at the time seems to have noticed it. But as an example of the way a common plant may at least become rare, I would mention some observations of mine on *Lantana mixta* in Singapore. When I first arrived in Singapore in 1888, this shrub was very abundant all over the waste ground in Singapore; on my last visit there it had become comparatively scarce—one had to look about the country for it. I found a plant in the edge of the wood by the roadside, and on examining it found that every one of the young fruits on the whole bush was perforated by a small green bug, and that all these fruits which had been sucked by the bug withered up and never came to maturity. Now as *Lantana* is widely dispersed by birds, which swallow the ripe seed and pass it unharmed, the occurrence of the bug and its attack on the young fruit consequently

entirely prevented the propagation of the plant. The bug appeared to me to be identical with a species which attacked the Cotton plant in the same manner, and was one of the two species which prevented our cultivation of Cotton in the Malay Peninsula. I will add that a large part of the *Lantana* which was destroyed throughout the island was destroyed by the extensive planting of Para Rubber in Singapore, whereby the lands which after the Chinese had abandoned the cultivation of Gambir and Pepper there had been largely covered with *Lantana* were again cleared of weeds and bushes, including the *Lantana*. The action of man in destroying species I will refer to later.

The action of enemies, whether insects, fungi, or bacteria, in the destruction of a species in a natural state has not yet received that attention of field naturalists which the subject requires; but every tropical agriculturist knows that an insect or fungus which commonly lives on one particular plant may adapt itself so as to attack another allied plant, and, if the latter grows in abundance in any area, may practically exterminate it.

An excellent example of the destruction of a species completely within a very limited space of time is furnished by the extermination of the two rats of Christmas Island.

This island was first visited by any naturalist in 1886, when the *Egeria*, with Mr. Lister as naturalist, visited the island. At that time the island swarmed with both species of rats, especially *Mus Macleari*, the white-tailed rat, of which, judging by accounts, there must have been millions. In 1904 Dr. Hanitsch and I visited the island to collect the flora and fauna, and though we went far into the forest and set traps for rats, not one of either species was to be met with. On inquiring of the residents on the island, we were informed that the white-tailed rats had totally disappeared, and the last one that was seen was rambling about apparently very sick in the neighbourhood of the settlement. What had happened? The only probable solution was that the destruction was caused by the introduction of the common brown rat; not from this animal having eaten up the food of the white-tailed rat, for the rat's food, the fruits of the trees in the island forests, lay untouched on the ground in abundance, but in all probability from the introduction of some bacterium (possibly the plague) by the stranger. The brown rat itself had not spread over the island to any extent, and mainly confined itself to the neighbourhood of the settlement.

A very similar case appears to have occurred also in the island of Fernando de Noronha, where, when this island was discovered, there was great abundance of a large rodent described by Mazarredo in 1774 as the *paca* or *mulita* of Buenos Aires. This animal has apparently utterly disappeared, for in my expedition to that island in 1887 we were unable to discover any trace of it, nor did any of the residents know of its existence. If such destructions of an animal species may occur in so short a time, why should not the same thing occur in plants?

The disappearance of *Hedychium coronarium* and *H. flavescens* in Ceylon and that of *Lantana mixta* in Singapore seem to show that this may occur.

DESTRUCTION OF SPECIES BY MAN.

The first botanist that came to Singapore, Dr. Wallich, in 1822 found the whole island densely covered by forest, and a few of a wild tribe of men known as the Orang Selitar lived chiefly in boats on the southern sea-shore. It was the custom, it appears, for ships travelling to the East to go through the Johor Straits, to avoid the pirate Orang Selitar and the pirates in Galang and the other islands to the south, which shows that the northern part of Singapore and South Johor were but little inhabited.

Wallich's collections were apparently chiefly made in the south of Singapore where the town now stands. Raffles later, to open up the island, introduced the Chinese, who felled for export a great deal of the timber trees and opened up, cleared, and burnt large areas for the cultivation of Pepper and Gambir, moving from place to place as they exhausted the soil and firewood. When I arrived in Singapore in 1888 only a very few forests remained in the island and the south of Johor. Over the rest of the island the forests had been replaced by Lalang grass (*Imperata cylindrica*), *Lantana mixta*, and other introduced plants, and by secondary growth. The sandy shores on the south were first planted with rice, then with coconuts; but a patch of the original flora of this area containing *Vaccinium*, *Melaleuca*, *Leucopogon*, and *Capparis Finlaysoniana* remained for some time more or less unaltered, but has since been destroyed, and as this was the only known spot for this beautiful *Capparis* it is probably quite extinct.

The remaining scraps of forest persisted, till a few years ago, under the control of the Forest Department, but were practically destroyed at length, with the exception of a few acres.

The demand for land for Rubber cultivation finished off most of them. The especially sought for trees valued for timber were naturally destroyed first, such trees, e.g., as *Dialium* and Dipterocarpeae. Where the ground was cleared on the edge of the forest, the bright sunlight let in destroyed speedily many of the delicate herbaceous plants like *Pentaphragma Ridleyi*, at one time very abundant on Bukit Timah, but which has been nearly exterminated by the making of a quarry and felling of timber which let the light into the rest of the forests. Grass fires on the jungle edge contributed to the destruction of the original flora within the forests.

My early collections here contained nearly all of the species collected by Wallich and Maingay, but many were then very rare, and some are now undoubtedly extinct. I will mention a few species only known from Singapore and now believed to be quite extinct. *Capparis Finlaysoniana* has already been mentioned.

Strophanthus Maingayi, Hook. fil. 'Climbing extensively over trees', Maingay. This beautiful plant, with white flowers as big as a wine-glass it is said, was again collected by Hullett at Changi before 1888. I visited the spot where he found it with him, but it was gone and has never been seen again anywhere.

Melastoma molle, Wall. Collected in Singapore by Wallich, 1822, reduced to a variety of *M. decemfidum*, Roxb., by Clarke, but certainly distinct, reported to have been obtained in Luzon by Cuming. I found a single small plant without any signs of flowers in dense jungle on Bukit Timah. The plant had disappeared on my next visit, and it has not been seen again anywhere.

Endopogon Ridleyi, Clarke. The whole of the country lying north-west of Bukit Timah has long been felled and cleared. It was being felled for timber in Wallace's time, 1854, as described by him in his 'Malay Archipelago'. There remained, however, a small patch of wood through which ran a small stream; on its banks grew abundance of *Endopogon Ridleyi*, and near it grew a fine Ginger, *Zingiber chryseum*, Ridley. In 1911 this patch of jungle was felled and burnt, and both species are probably now extinct.

Pinanga singaporensis, Ridl., grew in another patch of wood surrounded by extensive cultivated land. I have failed to find it anywhere else. Probably on account of the limited number of plants occurring here, and consequent want of cross-fertilization, it did not set fruit, and this wood was destroyed some years later.

Didymocarpus Perdita, Ridley. I found two plants of this on a bank in the centre of Singapore surrounded by extensive cultivation. It has never been seen again.

Euthemis minor, Jack., was described by him from Singapore, apparently common in 1820-2. After much search I discovered a single patch in the farther corner of Singapore. It is a sea-coast plant of the south of Singapore, but the greater part of the sea-coasts have long been cleared of their endemic vegetation.

Close to the Botanic Gardens stand no less than three unique trees, the remains of high forest formerly covering the ground, left because they were too high to destroy. Usually in this case the tree soon dies because of the exposure of its roots to the hot sun; these trees being better placed have survived. They are *Shorea gibbosa*, Brandis, *Parishia* sp., a male tree only, and *Ormosia macrodisca*, Bak.; this latter is said to occur in Sumatra, and there is a tree in the Buitenzorg Gardens. Otherwise no other trees of these species are known, and, as so often happens in cases of isolated trees, they failed to produce any healthy offspring.

In 1889 in the Kranji Mangrove swamps I found on the trees no less than forty-six species of Orchids in abundance. In 1915 I visited these swamps again to look for them, and could only find four or five of the

smallest kinds. The forest had been let for timber-cutting and the bigger trees bearing most of the Orchids cut out, and a patch of forest which shaded the edge of the swamp was destroyed, letting light and heat into the swamp.

The extermination of plants by man is not only effected by plantations on a large scale and destruction of the forests. Pulau Tawar is a Malay village of some size and antiquity on the Pahang river. On a visit there I observed that there were no Rattans (*Calami* and *Daemonorops*) in the neighbourhood, even seedlings, except one species of *Daemonorops* which had no value in the eyes of the Malays.

The Malays, who are insatiable for Rattans for house use and sale, cut the long stems when sufficiently developed, that is to say when they are big enough to flower. This constant cutting prevents the plant ever reproducing itself, and it is only a matter of a comparatively few years before the Rattans are utterly exterminated. The wild tribes, too, search the forests in which they live thoroughly for eatable *Dioscoreas* and Palms, as also for Rattans, Rubbers (*Willughbeia*), and destroy the big *Dipterocarpus* trees by boring them for wood-oil. Even if some few plants remain scattered here and there, they are apt to die out from want of cross-fertilization.

The extermination of species by man in the Malay Peninsula has really been only extensive within the last fifty years. It is very different in Ceylon, a heavily populated island for over 2,000 years. Any one who visits the forest country round Anuradjapura will be struck by the small size of the trees covering the area which many centuries ago was a thickly populated district. Here are the remains of temples which, with the houses of the inhabitants, must have been largely built with timber of the felled forests. What trees were they that were used for the beams and wood-work of the temples and houses? The valuable *Dipterocarpeae*, giving good timbers, are given in Trimen's Flora as six common, twenty-four rare or rather rare, twelve very rare, and four or five apparently extinct. Dr. Trimen points out the difficulty of obtaining specimens of these trees, and says our knowledge of the Ceylon species is very imperfect; but the numbers given above are about what one would expect of first-class timbers in a heavily populated country where timber of large size had been required for 2,000 years. It is now highly probable that these plants had formerly a very much larger area of extension. A large proportion of the plants labelled very common by Dr. Trimen are introduced weeds, and indeed, when in Peradeniya in 1888, I had to go a very long drive and walk before I got to a hill where the real Ceylon flora could be seen. Dr. Willis does not, so far as I can see, distinguish between the very common introduced plants and the very common indigenous plants. What were the plants on the ground before these weeds were introduced?

ALTERATIONS OF THE FLORA DUE TO CLIMATIC CHANGES.

A comparatively small change of climate may very easily cause the destruction of species on a large scale: (1) by actual destruction of species; (2) by allowing the development of an entirely fresh flora which would swamp the indigenous flora.

A diminution of the rainfall, for instance, due to excessive destruction of trees, could entirely destroy the epiphytic flora of a region. I have shown how on a small scale in the Mangrove swamps at Kranji in Singapore, and the felling of the jungle on the slopes of Bukit Timah, the light and heat let in destroyed the epiphytic flora and herbaceous plants in these forests.

I have another instance of such destruction. Some years ago, 1905, in Singapore, we had an extraordinarily dry and hot spell which lasted for a month or two, during which period the epiphytic Fern *Polypodium sinuosum*, of which there was a great abundance on some of the trees, was completely dried up, and almost every plant of it in the gardens utterly perished, and no young plants of it ever came up again on these trees.

At present we have very little information, at least collected together, of modification of climate and consequently of floras in a natural condition of things, and alterations due directly or indirectly to the action of man. These are subjects well worthy the study of naturalists. Changes of climate have occurred without any geologic cataclysm in past years we know, e. g. the glacial period of Europe, and I understand the desert period of the south of England. In Nicol's 'Three Voyages of a Naturalist', chap. iv, in describing South Trinidad, he notices great numbers of standing and fallen trees apparently dead for many years covering the whole island, and the disappearance too of the goats which formerly inhabited it. There is no clue as to the cause of the destruction or its date, and he considers it improbable that it was caused by volcanic action, as at the summit trees and tree-ferns still flourish, and there are no traces of fire. However, as it is a volcanic island, the destruction might have been caused by the emission of the poisonous gases or water, as was reported to me as occurring in the Hawaii islands some years ago.

That floras do change without human interference we know from Clement Reid's researches into the early floras of Britain and Holland, species entirely disappearing and being replaced by others, but from what causes we are still ignorant. There is no evidence whatever to show geological cataclysms in all such cases. The process may be and probably is slow at most periods.

ENDEMIC SPECIES.

An endemic species or genus is one confined, so far as is known, to one definite area, and is not known to occur outside that area. Such a species may be one which has evolved within that area, and for some reason never spread farther, or it may have at one time or other occupied a wider area from which, except in one region or locality, it has disappeared. Before we can say definitely a plant is endemic we must have a complete knowledge of the flora of the nearest countries, but this in many cases we do not possess. Thus, though there are a very large number of what have to be recorded as endemic plants in the Malay Peninsula, we know comparatively little of the floras of the adjacent countries, Sumatra, Borneo, and the islands south of Singapore, Siam, and Cochin China. The nearest land to Ceylon is southern India, Madura, and the Carnatic. Has this region been so thoroughly explored that we can say for certain that many of these so-called Ceylon endemics do not still occur there? I doubt it very much. This part of India has been very long heavily cultivated and thickly populated. How many of the now endemic plants of Ceylon were not formerly abundant over this area?

Dr. Willis himself, on p. 12 of his paper in these Annals, gives an important clue to the history of endemics in Ceylon when he says, 'The second point that shows at once in these diagrams is that the enormous majority of the endemic species are in the wet zone'. Exactly what would be expected if the climate of southern India and the remainder of Ceylon had been formerly a wet district like the Malay Peninsula, and by land changes, denudation of mountains, felling of forests, changing rainfall or other climatic changes had become xerophytic except in the still wet zone of the south-western quarter of the island. In that case we should find exactly what we do find—the remains of an old rain-forest flora isolated in the wet zone.

We find evidence of a reverse action curiously in the case of the limestone rocks of Selangor in the Malay Peninsula. These rocks, attaining a considerable altitude (about 1,000 feet), lie at present thirty-two miles from the sea, though there is still a tradition of the sea having washed their bases in the time of man. Between them and the sea is a flat area of wet rain-forest which has crept up their sides for some distance. At the top of these precipitous rocks there is much mica in the soil, which must have come from granite mountains in close proximity to the limestone and higher than it. These mountains are now gone. On the top of the limestone rocks we get a distinct flora largely identical with that of the Tenasserim limestone, including two species of *Boea* closely allied to plants of Tenasserim and Borneo, and *Calanthe vestita*, only known from the limestone rocks of Tenasserim and Borneo. Here we have the remains of a xerophytic flora

persisting on the wet limestone rocks, surrounded by a geologically modern rain-forest flora, which has swamped it everywhere but on the summit where the big rain-forest trees could not grow.

'Endemic species confined to small areas are really species in the earlier stages of spreading, and, given time enough, they might ultimately be found covering large areas. Endemic species begin as VR in some given country, and gradually extend their area, passing upwards through the stages R, RR, RC, &c.'¹

This general statement could only be correct if these endemics were found to have all arisen from other species now in the island. Dr. Willis gives a list of genera containing endemic species, but omits all the endemic monotypic genera, *Trichadenia*, *Fulostyles*, *Pityranthe*, *Pseudocarapa*, *Glennia*, *Pericopsis*, *Schizostigma*, and many others, and does not mention the fact that a large number of the endemic species are the only ones of their genus in the island. How can these be species in the early stages of spreading? Furthermore, most of them are rare, and some almost, if not now quite, extinct.

On examining the affinities of these endemics we find that a very considerable proportion are Malayan. Now the connexion of the Malay region with Ceylon must have been at a very long distance of time ago. The genera containing endemics are nearly all rain-forest country plants, and appear to be now nearly confined to the wettest region of Ceylon. Exactly what we should expect if there was at a very long distance of time a land connexion with the tropical rain-forest region, which being destroyed, this very old flora persisted in the wet mountain regions till a large portion of it was destroyed by man, directly or indirectly, by felling the forests and causing diminution of the rainfall over a large area. This will account for the state of the flora and its constituents, but Dr. Willis's theory will not.

'Ceylon, though equatorial in position' (which it is not), 'has but a small flora (2,809 species) compared with the islands of the eastern peninsula of India. . . . This has always been a difficult matter to explain, and the Natural Selectionists have had two rival hypotheses. . . . The first is that Ceylon has a less "tropical" climate than Malaya, having greater extremes of wetness and dryness and of heat and cold. The second is that Ceylon has but a poor soil, . . . it being all the product of the decay of gneiss and granite.'² I do not see how these two theories are 'mutually contradictory', nor do I know who the Natural Selectionists are who have made these suggestions. It is obvious to every one who has at all examined the flora of Ceylon, that, as already pointed out, an immense quantity of the original flora has been destroyed by man but replaced by imported weeds which largely bulk in the VC's of Trimen's Flora. This destruction was

¹ Ann. Bot., L. c., p. 5.

² L. c., p. 21.

going on as late as 1900 (see Brown's article on the Forests in the Appendix to Trimen's Flora). A very large proportion of the species in the Malay region are epiphytes, especially Orchids. These are the first to disappear when timber is felled and the country exposed to the sun. The very small number in the plain districts of India illustrates this. In Ceylon there are only eighty species against over 130 in Singapore island alone.

THE MUTATION THEORY.

The theory of the evolution of species by Natural Selection is, as I understand it, as follows:

An organism produces forms which in various ways are not identical with the parent form. These forms are known as varieties or mutations. Should any of these forms be adapted in any way so as to be more suitable for the surrounding conditions than the parent form, they may persist and reproduce themselves in the mutation form. The mutations do not necessarily reproduce their replica, but may revert to the original form. If, however, selection comes into play and the mutation is continually selected, the form becomes a fixed mutation. I shall give instances in the case of *Antigonon* and other plants.

Should the forms connecting the fixed mutation with the original form disappear, and the alterations be sufficiently distinct and important to warrant it, we call the new form a species.

For a mutation to become a fixed one it is necessary that it should be able to reproduce itself successfully and continuously.

In most or more probably in all cases of successful fixed mutations the determining factor is the surrounding conditions environing the original plant. Thus a plant adapted for the dense shade of the forest may disseminate its seed nearer the edge where the light is greater. Here natural selection comes into play, and the mutations more adapted for greater light will grow and reproduce along the edge. If the forest be near the sea, the mutations gradually getting nearer and more adapted for sandier and sandier spots may in time be so selected that they take on a maritime form. This form in time becomes so far adapted to a littoral life that it cannot revert to the jungle form, and remains as a species.

This is only one sample of the evolution by Natural Selection; others are connected with adaptations for climate, fertilization, dissemination, &c., the object being that an organism can by mutating fill up a space, or state of conditions, which in its original form it cannot do.

This theory accounts for the great number of species in the world and their adaptation to their surroundings and conditions of life, and can be tested and proved by a study of mutations. No other theory has been produced which can account for the facts.

Dr. Willis's theory of mutation is described in the 'Annals of the Roy. Bot. Gard., Peradeniya,' as that new characters are supposed to arise at one step: once they have appeared the new characters are hereditary and the new form does not go back to the old one.

In other words, all mutations are at once fixed. This is easily shown not to be the case. Nor does the theory in the least explain the adaptations to surroundings, e.g. why *Calophyllum inophyllum*, adapted as its fruits are for sea dispersal, occurs only on the sea-shore, or why *Crinum asiaticum*, with long-tubed white fragrant flowers only fertilizable by a crepuscular sphingid, only opens its flowers at exactly the time of the appearance of the moth. In fact, the theory is the old special creation hypothesis with the creator left out, and no substitute given.

Every gardener and field botanist of ordinary observing powers knows that very many variations (or mutations as Dr. Willis prefers to call them) occur in plants which do not appear again in their offspring.

Many years ago in Singapore we obtained a variety of *Antigonon leptopus* in which the normally pink flowers were pure white. This variety, though it came true from cuttings, did not come true from seeds. All its seedlings gave pink flowers for a considerable period. After some years (the mutation not being fixed till then) we obtained a few white-flowered seedlings. Zinnias grown in the garden at Singapore from cultivated plants from Europe in three years reverted to the form of the original small-flowered wild plant. The same thing happened with the garden *Balsam* and other cultivated plants. But this is well known to every gardener. Very striking cases in a natural state will be shown later on in this paper.

The argument that specific differences which plants possess have never been shown to be of practical use now or of some use in the past, does not hold good in view of the extensive literature on fertilization and dissemination, and on the relations of plants to their surroundings.

The whole life-history of any single plant is not at present really known—its physiology and habits; its insect, fungal, or other enemies; its requirements due to the action of light, heat, electricity, rain, dew, frost or drought; its food and water-supply; its means of fertilization, protection, and reproduction; in fact, the whole physiological and ecological history of the plant from the seed to its reproduction and death by day and night, in normal and abnormal weather, at all seasons of the year, and in all geological or climatic changes for the period of its existence as a species. Until this is known, it is impossible to give the cause of all specific differences. I do not believe this is known of any common English plant, still less of any tropical one.

In Ann. Roy. Bot. Gard., Perad., vol. iv, p. 2, Dr. Willis writes: 'A point that has so far escaped attention is, that the characters that distinguish genera

and species are largely characters of the floral organs; the struggle for existence is almost entirely among the seedlings and young plants in which these organs are not present. To take an example, is it conceivable that in *Dillenia* it can make any difference whether these leaves are acute or obtuse, or the petiole one inch or one and a half inches long? These are the only characters that can show till the plants are at least ten years old, by which all that are going to die out will have done so.' But the struggle for existence does not cease in any plant when it has developed out of the seedling stage. It is surely well known that the struggle continues throughout the whole life-history of the species. I need not, however, go into this, as plenty has been written to show this both by Darwin and later botanists.

The lengthening of the petiole and acute or blunt apex of the leaf may have the utmost importance to the *Dillenia* in the relations of the leaf to sun and rain. But I will give one or two examples of 'infinitesimal variations' which have the greatest importance to the life and propagation of a plant.

Metroxylon Sagus, Rottb., and *M. Rumphii*, Mart., the two Sago Palms, are very closely allied plants, but the latter is armed with spines especially on the leaf sheaths, the former is unarmed. In parts of Borneo it is impossible to cultivate successfully *M. Sagus*, because the wild pigs (*Sus barbatus*) attack and devour the young shoots as fast as they come up. *M. Rumphii*, guarded by its spines, is immune from the attacks of this animal. Here the development of spines protects the spiny Sago, which would otherwise be exterminated as the smooth Sago is. Another instance of a totally different character is shown in the case of the Macarangas, as described in my paper on Ants and Plants in the 'Annals of Botany', vol. xxiv, p. 471, where it is shown that in certain species of the genus the young leaves are liable to so serious an attack by caterpillars that the plant may be severely injured, if not killed; the very slight variation of the longer persistence of the bud sheaths for some days, and the development from the glands (common in most species of the genus) of food bodies attractive to ants, by inducing ants to take up their abode in the hollow stem, protect the plant from caterpillar attacks.

Castilloa elastica in the Malay Peninsula is attacked by a beetle, *Epepseotes luscus*, the larva of which tunnels the stem, causing the destruction of the tree. The insect can only escape through the scar of a fallen leaf, as it is the only part of the trunk not guarded by lacticiferous vessels through which it cannot pass. A comparatively slight thickening or hardening of the texture of this point would effectually stop the beetle from escaping, and render the tree invulnerable; and from some such slight variation I found trees immune from the attack of this beetle, while others standing close by were destroyed.

I cultivated in Singapore two kinds of Lilies, of which one, *Lilium*

auratum, had narrow, the other—I think *L. croceum*—slightly broader leaves. Rain falling on the latter was retained round the bud by the broader leaves, which formed a kind of cup, the buds were destroyed, and the plant failed to flower. In the other species, the narrow leaves did not meet at the edges, and through the space between the narrow leaves the rain-drop fell and the buds were uninjured, and the plants flowered.

A most amusing passage occurs in one article, showing, I think, a want of careful thought by the author: 'Can it for instance be supposed that the hereditary fasciation of the Cockscomb is of any use to that form?' To this I reply it certainly can, for specimens that are not fasciated (which do not rarely appear) are worthless from a gardener's point of view, and quickly find their way to the garden bonfire.

A very common variation occurs in the form of variegated leaves, which are blotched or streaked with white. Now every gardener knows that this mutation is often very unstable, and the plant readily reverts to the original green-leaved form. There is a variegated form of *Arundo Donax* very commonly cultivated with leaves edged or otherwise marked with white; when cultivated for some time the plant produces branches bearing typical green leaves. If these are not removed, in a few years the whole plant reverts to the original plain green colour. Here is a case of reversion of a mutation which Dr. Willis states does not exist. Let us compare this with the Aroid *Aglaonema costatum*. This plant has three forms—one with blackish green leaves mottled with white, one with dark green leaves with a central white midrib, and one with light green leaves with white spots. These three forms grow in limestone districts north of the Malay Peninsula. Unlike the *Arundo* they do not revert, but each variety reproduces the same form. No variety with plain green leaves has been seen. Both of the first two grow in the same area, the third farther north in southern Siam. Here are variations which keep true under any form of cultivation, and would be cited by Dr. Willis as proofs of his theory. But if so, it would be incumbent on him to show why the *Aglaonema* comes true in all its three forms, and why the *Arundo* does not. Here his theory completely breaks down.

Now it is well known that it is in limestone districts that one always finds the largest number of plants with white variegated leaves. Near the limestone districts in Sarawak I went one day for a stroll and collected a single plant of everything I could find with variegated leaves. In an hour I had my arms quite full of variegated plants of many different orders. The reason for this is quite obscure to me, but it is clear that some advantage must be gained to the plant by this variegation, and that it is of so much importance to the plant that it is a permanently fixed mutation. Why are other plants similarly variegated not equally permanently fixed as a variation, as they should be by Dr. Willis's theory? Another instance. The Tahan

river is a rapid running, rocky mountain stream flowing through the forests. Along the banks of this stream I found a whole series of plants which possessed long narrow leaves of willow-leaf shape. They included species of *Calophyllum* (Guttiferae), *Ixora* (Rubiaceae), *Hygrophila* (Acanthaceae), *Didymocarpus* (Gesneraceae), *Podochilus* (Orchideae), *Antidesma* (Euphorbiaceae), *Ficus* (Urticaceae), *Melastoma* (Melastomaceae), *Rhyncophylla* (Aroideae). These fringe the rocks and are often submerged by a very violent torrent. All or almost all are allied to species living out of the reach of the water, with broad lanceolate leaves. Should these broad-leaved plants be subjected to the rush of the torrent, their leaves would be torn to bits and the plants destroyed. But the form of the leaves varies occasionally, e.g. in *Ixora*, farther in the forest, we get forms with more narrow lanceolate leaves. According to the theory of natural selection by variation, the *Ixoras* with lanceolate leaves could establish themselves along the stream edge more readily than the broad-leaved ones which have their leaves destroyed by the torrent. A variation with narrow leaves could approach nearer the edge where the rush is more violent, and so it could go on till we found, as we do among the rocks where the torrent at certain times is excessively furious, the *Ixora* growing with foliage more like that of a stream-water plant, thriving and holding on with a mass of strong woody roots, and a tough short stem, with none of its leaves injured or its boughs broken. The theory of Natural Selection by infinitesimal variations will account for this, but the mutation theory will not.

On the sandy shores of the Pahang river grows a species of *Vitex*, a prostrate creeping shrub, throwing up short branches four to six inches tall, with simple ovate blunt leaves 1 to 1.10 in. long; the flower spikes are a little over an inch long, with rather showy blue flowers. As this looked likely to prove an ornamental plant suitable for bedding, I brought some to Singapore, where it immediately turned into *Vitex trifolia*, an erect shrub or small tree about 10 feet tall with trifoliate leaves; the leaflets obovate acute or elliptic, 2.5 in. long, 1 in. wide, with a petiole 0.4 in. long, and raceme or panicle of smaller flowers 6 inches long. The sea-shore form was abundant and occurs elsewhere, and would certainly be considered a distinct species, differing as it does importantly in all its parts—stem, leaves, inflorescence, and flowers. Yet Dr. Willis says that 'no evidence has ever been brought forward to prove that local species are adapted to local conditions; it is simply an hypothesis' (Ann. Bot., l. c., p. 15).

Microcarpea muscosa, R. Br., is a small scrophularineous plant which grows on the edges of ponds. Where the water subsides and the plant is left on the bank it can be seen to be erect, 3-4 inches tall with little violet flowers, but beneath the water it forms large, short tufts, and the corolla scarcely projects beyond the calyx, the limb of it, almost reduced to a rudiment, bearing mere traces of its violet colour. There are numerous instances of similar

occurrences recorded by botanists, and I would especially refer Dr. Willis to Hiern's paper on 'Forms of Floating Leaves' (Camb. Phil. Soc., xiii), and call his attention to the well-known variations in *Polygonum amphibium*.

The whole meaning of these adaptations of local species to local conditions is not always clear. I do not clearly see why, for instance, the Alpine plants of the high mountains of the Malay Peninsula and Ceylon have a tendency to the reduction of the whole plant in size, the thickening of the leaves, and their having a tendency to become eventually quite round, orbicular in fact, and in the case of compound leaves, simple, a state of affairs which does not occur in any of the species of the same genus in the wet forests at the base of the mountains; but there cannot be a shadow of doubt that it is of importance to the plant, and that the plants have been gradually adapted to their surroundings. Dr. Willis seems to be puzzled as to why different species of a genus occur in the same region. Why, he asks, in the 'Annals of the Roy. Bot. Garden, Peradeniya,' vol. iv, p. 11—why should *Dillenia retusa* with its obtuse leaves and small flowers be found alongside *D. indica* with acute leaves and large flowers? In the eastern peninsula again live *D. ovata*, *D. meliosmaefolia*, as well as *D. indica*. Why should they be better suited to the eastern peninsula while *D. bracteata* suits Mysore and *D. retusa* Ceylon? I have not seen *D. retusa* or *D. bracteata*, and we must add *D. dentata*, Thunb. (*Wormia triquetra*, which is a true *Dillenia*), growing wild, so I can give no answer as regards them, but the story of the Dilleniaceae of the Malay Peninsula I can at least partly give.

D. indica is exclusively confined to wet ground, chiefly river banks. It would not grow in the ordinary soil of the gardens of Singapore, but planted on the edge of ponds and in swampy grounds it did well. Its fruits are very large and round with immensely thickened and enlarged sepals, and measure 4-6 in. through. These sepals are not sweet to taste, nor is the fruit eaten by any animal. It is adapted for dispersal by river. River and sea-dispersed fruits have a tendency to become very large, e.g. *Calophyllum macrocarpum*, *Barringtonia speciosa*, *Cerbera Odollam*, &c., and this entails in many cases a corresponding enlargement of the flower, well seen in *D. indica*. *D. ovata*, Wall., and *D. aurea*, Sm., inhabit dry, xerophytic spots in rocky or sandy localities. They have bright yellow flowers and smaller-sized fruits; both are rare in the peninsula, as the climate, except in the north, is unsuitable. They appear to have originated in a dry region and have pushed a short way into the peninsula from Siam. *D. meliosmaefolia*, Hook. fil., is a small jungle tree occurring in our wet forests. To adapt it for that life the leaves and shoots are densely hairy, to throw off the rain which otherwise would injure the leaves. Hairy leaves such as these I find dry after rain quicker than smooth ones, besides retaining less water on the leaf during a shower. The leaves and buds of the trees in the forests are all guarded in various ways from the action of rain, or rather from the action

of the sun's rays after a rainfall, which burns the leaves. This hairiness is one system. The flowers are small and yellow; the fruit is orange-coloured, sweet, and eatable. It is small, hardly an inch through, so that birds can easily swallow it, and so disperse the seeds. It is obvious that a tree habituated to live in thick tropical rain forests would not thrive in a xerophytic country like Ceylon, though should it get there possibly it might exist in the small wet area. *D. Scortechinii* is a tall jungle tree attaining a height of upwards of one hundred feet; its flowers appear never to possess any petals, and it seems to be self-fertilized. Owing to its great height and comparative rareness of flowering I have not been able to make many observations on it. Like *meliosmaefolia* it inhabits wet jungles, but being a lofty tree its coma of foliage gets a full supply of light, and probably by reason of its being exposed to wind its leaves are able to dry faster and are not liable to injury from rain and sun. The fruits are of the same size as those of *meliosmaefolia*, but are green, glabrous, and not sweet. They appear to be dispersed by rolling and possibly by squirrels, rats, or bats. The absence of colour shows that they are not intended for dispersal by birds.

Now here we have four or five species in this one region, but the whole of their life stories are quite different, and they do not grow alongside each other as Dr. Willis says of the Ceylon species. Do the other two species in Ceylon grow in the same situation and surroundings as *D. indica*? We have no evidence from him or from Dr. Trimen that they do. Dr. Trimen describes the fruit of *D. retusa* as 1-1½ in. through, and orange. This suggests at once that it is bird-dispersed. The fruit of *D. dentata* is small and apparently whitish green. Both these plants require careful study in the field before we can say anything more definite about them. In the genus *Calophyllum* he states, p. 13, that an endemic species, *C. parviflorum*, lives beside the almost cosmopolitan species of the eastern tropics, *C. inophyllum*. The two are very much alike, but the latter has a globose fruit, the former an oblong, rostrate one. The latter lives in beach-forests, the former more inland (how then can they be living beside each other?). Now is it to be supposed that the shape of the fruit can have any effect upon the life of the species sufficient to account for its being evolved by a natural selection of infinitesimal variations, though it may be correlated with some internal character fitting it for life more inland?

I am met at once with a difficulty: I cannot find any species of *Calophyllum* named *parviflorum* except a species of Bojer's from Mauritius, but I must take it that Dr. Willis refers to one of the other ten species recorded in Dr. Trimen's Flora. It is probably *C. Burmanni* to which he refers, as it occurs in the low country near the sea and has more or less oblong fruit, though that species differs from *C. inophyllum* in almost every other part of the plant, including the flowers, for it is apetalous. Indeed there is no species in Ceylon which really resembles *C. inophyllum* except in general character,

Now *C. inophyllum* is a very peculiar tree ; it grows to a large size on sea-shores in sand, and its fruits are adapted specially for sea-dispersal. The fruit is green, globose, and resinous ; the endocarp is thin and woody ; it is adapted by its lightness for sea-dispersal, and its wide distribution is due to this. It is easily nibbled through by rats and squirrels which destroy the seed, a catastrophe not likely to occur to any extent in its native habitat, where these animals are scarce ; besides, owing to the globose form of the fruit, on falling from the tree it usually rolls at once down the sloping shore into the water and is drifted out of the reach of the animals. In trees in the Botanic Gardens at Singapore, however, the seeds were destroyed in hundreds by these animals ; in one year, at least, hardly one escaped. The fruit, on account perhaps of the resin, and certainly on account of its size, is not swallowed by birds or eaten by bats so far as I have seen. The tree does not thrive at any distance from the sea, and does not propagate to any extent in scrub or thick forest. It is not to the tree's interest therefore that birds or bats should carry its seeds away inland.

In *C. Burmanni* we find the fruit is half an inch or little more long and bright orange-coloured. This colouring is unusual in *Calophyllum*, and with its size suggests that the plant is bird-dispersed. Most of this class of fruit are green or purplish and seem to be dispersed by fruit-bats. *C. inophyllum*, being a maritime plant adapted for inhabiting sea-shores, was in all probability evolved in the Polynesian islands, and has been distributed by sea as far as the Mascarene islands. The head-quarters of the other inland *Calophyllums* is undoubtedly the Malay Archipelago and peninsula. The Ceylon flora possesses a considerable element of Malay forest plants ; indeed, nearly all the Ceylon Guttiferae have Malay affinities and all the genera and some of the actual species are Malayan. All the evidence of this order as of others goes to prove a former land connexion with the Malayan region. In this case it is clear that the inland *Calophyllums* may have invaded Ceylon by land at a very early date, while *C. inophyllum*, which occurs practically on every sea-coast it could grow on between Polynesia and the Mascarene islands, came by sea, perhaps hundreds or thousands of years later, and, as the seeds are still commonly to be found drifting along in the sea, may be still landing on the Ceylon shores. What then is remarkable in finding *C. Burmanni* and *C. inophyllum* in the same area ? and what bearing has the one on the other ?

It is unfortunate that Dr. Willis seems to base so many of his arguments on statements made in books rather than on observations made in the field ; thus he states (Ann. Roy. Bot. Gard., Perad., iv. 2) that *Ramunculus bulbosus* differs from *R. repens* mainly in the fact that the former has the sepals reflexed and the latter has them spreading ; but surely the bulbous root of one and the stoloniferous habit of the other are important differences, not to mention differences in the leaves. In vol. iv, p. 69, he gives a study of the tree

Dilleniaceae of the East as illustrating his mutation theory, which study is based, I imagine, on the first volume of Hooker's *Flora of British India*. He states that *Tetracera laevis* is found in Ceylon, Malabar, and in Java and Borneo. This species is closely allied to *T. assa*, and is peculiar to Ceylon and Malabar, and does not occur elsewhere so far as is known, the other localities quoted being erroneous. *Delima laevis*, he says, has only been collected in Malacca. 'If we accept the theory of infinitesimal variations we must either admit that *D. sarmentosa* was evolved near Malacca and afterwards spread enormously, while *D. laevis* has not spread, or else that there has been a vast amount of destruction, reducing *D. laevis* to one locality or destroying the other species that were evolved with it. It is, however, very nearly allied to *D. sarmentosa*. The small spread of *D. laevis* is easily accounted for on the mutation theory, for it may have been quite recently evolved, and not having a very efficient distribution mechanism would not travel very far except in a great length of time.' An examination of the type specimen of *Delima laevis* in the Kew Herbarium shows that it is *Tetracera borneensis*, Miq., a well-known and not very rare species, which has no connexion with *Delima sarmentosa* at all.

Of *Wormia* he writes (p. 72): '*Wormia triquetra* is confined to Ceylon; it belongs to the sub-genus *Eu-Wormia*, to which also belong *W. pulchella*, *W. meliosmaefolia*, *W. Scortechinii*, and *W. Kunstleri*. All the last three may be supposed to have split off from *W. pulchella*.' Now strange as it may appear, *Wormia Scortechinii* and *W. Kunstleri* are the same species, and with *W. meliosmaefolia* are not *Wormias* at all but *Dillénias*, and have no connexion with *Wormia pulchella*, a true *Wormia*. Further *Wormia triquetra*, from Dr. Trimen's description and figure and specimens preserved at Kew, is also a *Dillenia*—*D. dentata*, Thunb. The two genera are distinguished by their fruit, those of *Dillenia* being indehiscent with no aril to the seeds; those of *Wormia* opening out into a rose-shaped pink or white circle of carpels widely dehiscing and exhibiting the seeds invested by a scarlet aril.

I confess I do not understand Dr. Willis's interpretation of the simple facts of evolution of a species as generally understood by naturalists. He states that the idea that endemic species were evolved to suit local conditions is based largely upon Wallace. Who ever possessed such a curious idea? Let us take some examples of endemics. *Aphyllanthes monspeliensis* is a liliaceous plant occurring in a limited area on the Mediterranean where it is distinctly endemic, a monotypic genus of the Sowerbieae section with no relations nearer than Australia. *Helxine Solerolit*, Req. (Urticaceae), is an endemic of Corsica and Sardinia and has no relations at all in this region. *Dioscorea pyrenaica*, Bub., is endemic to the Pyrenees and the only species of its genus in Europe. How could these plants be evolved to suit local conditions, unless they are the relics of floras of which the rest is extinct?

There is nothing within thousands of miles of them now from which they could be evolved.

In cases like this, and there are very many, his mutation theory utterly collapses.

But what really puzzles him is the case of endemic species apparently actually evolved on the spot, such as *Castelnavia*, the various locally evolved *Eugenias*, *Sonerilas*, *Impatiens*, and such genera where one finds a number of peculiar species in one area. To assume that because we have not worked out the whole life-histories and conditions of environment of all these different species, their specific differences cannot be due to local differences in condition, and they must have been produced by special creation, for that is what his mutation theory amounts to, is hardly scientific.

In most cases of mutation and especially where one organ is much altered, other organs are also modified; e.g. a dwarfed plant of *Liparis longipes* was imported into Kew Gardens many years ago, in which not only the pseudo-bulbs but the leaves and inflorescence and all the organs of the flower down to the lip were shortened and broadened. Another example is the case of the *Vitex trifolia* previously described, where, though almost every organ in the littoral form was altered in shape, the plant proved to be not even a fixed mutation.

Species absolutely isolated, either by the extinction of all allied forms or by accidental position, do not produce a series of mutations.

There are only two species of the order Dioscoreaceae in Europe at the present day, the plants of this order being chiefly tropical. These are *Dioscorea pyrenaica* of the Pyrenees and *Tamus communis*. Though these plants must have inhabited Europe for an immense series of years, being undoubtedly the relics of an early tropical era, they have not produced yet anything that could be called a mutation. The same remark applies to *Aphyllanthes monspeliensis*, and many other instances might be deduced. A great contrast to this is the case of such genera as *Hieracium*, *Rubus*, *Rosa*, and *Saxifraga*, in which genera the number of mutations is very large. The same phenomenon occurs in all parts of the world. I would call attention to the endemic plants in Christmas Island in this connexion. This island has never had any connexion with any other land, being a volcanic island dating, it is believed, from the Eocene period. The flora which had not been interfered with by man, when it was first examined, consisted of plants nearly all of Javanese affinity; many were sea-shore species of wide distribution. The endemic flowering plants are thirty-one in number, all belonging to separate genera, except two *Grewias*, two *Phreatias*, and two *Pandani*. Here, in spite of the long period during which this island has been above water and capable of bearing a flora, there has been no evolution of a large number of any one species comparable with

the evolution in *Hieracium* or *Rubus*, nor even to the Grewias of the Malay region or the Pandani of Madagascar.

Again, in Europe we have of Gesneraceae, *Ramondia pyrenaica*, Lam. (Pyrenees), *R. serbica*, Panc. (Serbia), *R. Heldreichii*, Boiss. (Thessaly), *Haberlea rhodopensis*, Friv. (Thrace).

These grow in much the same kind of locality as the Saxifrages do, but what a contrast! Of Saxifrages in Europe we have over a hundred species and innumerable forms. The nearest Gesneraceae to our four European species are in tropical Africa¹ and the Himalayas, while the Saxifrages range over the whole of the north temperate zone and even South Africa, Australasia, and South America. Now look at the Gesneraceae of the East Asiatic tropics; of *Didymocarpus* we have over fifty species in the Malay Peninsula alone; the closely allied *Chirita*, 7, *Loxocarpus*, 4, *Paraboea*, 13, *Boea*, 12. We find a large number more of *Didymocarpus* in India, Borneo, Java, and Sumatra; *Chiritas*, about fifty more in Cochin China, Assam, Ceylon, Java; *Loxocarpus*, several in Borneo; *Paraboea* in Siam and Assam; *Boea* in Borneo, Java, Siam, and Cambodia. All these countries are in actual land contact with the Malay Peninsula, or were formerly so. They differ much in climate and soils.

Let us assume that a species of *Didymocarpus* has been evolved in the Malay region, and spread gradually as far as the countries and localities would admit of its growing. In various spots, and perhaps distant parts of these countries, mutations formed, and became fixed mutations or what we should call species. A species is formed, let us say, on the Tenasserim limestone rocks; it pushes down along the limestone chain again to the Malay Peninsula. Here it meets with allied species with which it can cross, and a fresh series of mutations is formed, some of which, adapted to special circumstances, can live and thrive on spots where the original species could not.

We have the European Gesneraceae and Dioscoreaceae, and *Aphyllanthes*, all isolated species, with no relatives near enough to hybridize and cause the production of an extensive evolution.

We have *Saxifraga* in Central Europe, in Arctic Europe, the whole of the temperate regions of Asia and America. The wave of cold in the glacial period must have driven out many species in Europe, but there were mutations which had adapted themselves to the cold and survived; and these returning hybridized with the species surviving in the warmer valleys of the south and started a new series of mutations. *Hieracium*, *Rubus*, *Rosa* have all the same distribution and story.

But the European Gesneraceae and Dioscoreaceae and Sowerbieae (*Aphyllanthes*), the relics of an old long-lost flora, isolated by thousands of miles from any allied species, remain unaltered, with no series of mutations.

¹ *Saintpaulia*.

Just in the same way in Ceylon we have isolated species like the Dipterocarpeae and *Acrotrema*, a native of South India and of the Malay Peninsula, both countries formerly connected with Ceylon, and from both of which mutations or species may have come, and by crossing, formed the seven recorded species of *Acrotrema* (the largest number of species of the genus known anywhere) now inhabiting Ceylon. Indeed both Thwaites and Trimen considered it probable that some of these species were natural hybrids. I have only been able to outline this suggested cause of the state of affairs which the distribution of plants and the great preponderance of species in one group and the rarity of others seem to evidence. To give fuller instances and to give such evidence as is known would require a large-sized volume. Nor do I at all intend to intimate that this alone accounts for the immense number of species and genera we see in the world, and the facts of their comparative abundance or rarity. The whole subject is more complex than that.

It is perhaps as well to point out that it is not necessarily the change to another country that is the cause of fixation of mutations; every country, every district, has a variety of localities into which a plant may push by a suitable mutation. Given just the similar conditions for the plant, there is no probability of any mutation for the form which finds its way there being in any way more suitable, but where conditions are different, a mutation even slightly more suitable would be necessary for the plant to establish itself. Thus *Ixora Lobtii*, Loud., is a tall, broad, oblong, lanceolate-leaved shrub, the leaves about two inches across. It grows in thick forest, where, owing to the comparatively scanty sunlight, it requires broad leaves to receive a sufficient supply of light. Through the forest runs the rapid stream Tahan. The seeds of the *Ixora* (dispersed by birds) must frequently be dropped nearer the river edge. The vegetation is thinner here, and there is more light. At certain times the river rushes in spate through the wood-edge, and a narrow-leaved form would be less injured by the water-rush than a broad-leaved one. On the actual rocky edge of the stream the rush is much more violent and constant, and only very narrow leaves can stand against it. Gradually, by eliminating the wider-leaved mutations, there appear forms with very narrow willow-like leaves, var. *salicifolia*. This edge of the river is in bright sunlight, so that the advantage of broad leaves to the plant is lost. Now suppose a fall of the lofty hill-side so as to block up and divert the Tahan river, leaving the *Ixora* in an area quickly covered by dense forest. Owing to the reduction of sunlight, the narrow leaves are now an injurious mutation, the plants with slightly wider leaves would supplant those with willow leaves, and eventually by the elimination of the narrow-leaved forms the plant would revert to the jungle form.

The 'struggle for existence' is not of course merely the struggle

for life of an individual or species, it is the struggle for successful continuous propagation." In most cases thousands of the seeds produced are absolutely wasted, only those are not which find a suitable spot for their future reproduction. A species modified for life in the full sun of a heath may, increasing the width of its leaves, first push into the thin scrub of the forest edge, and, gradually modified, may eventually become adapted for life in the dark, wet forest; or it may modify itself to grow in salt mud, swamp, the sand of the shore, or on coral rocks or on mountain tops.

VERY COMMON PLANTS.

On p. 17 of Ann. Bot., vol. xxx, Dr. Willis in dealing with very common plants writes: 'Why should a species that ranges over Ceylon and Peninsular India be commoner in Ceylon than one that only ranges over Ceylon?' and makes the advocates (of Natural Selection I suppose) reply: 'Because it has a wider range.' This, he says, is simply an appeal to ignorance; and here I heartily agree with him, merely suggesting he should refer the question to an observer of plants in place of such advocates. His suggestion is, that the reason why mere wide range should involve commonness is their greater age within the country.

Let us examine the story of some common plants, and first take *Imperata cylindrica*, the Grass known as Lalang in Malay and as Illuk in Ceylon. It was, when I first reached the Malay Peninsula, the commonest plant in the peninsula. There was over large areas much more of it in bulk than any other plant, and far more than of any indigenous Grass in the whole peninsula.

It is generally believed to be a native of Africa, and was probably introduced somewhere about 1822 into the Straits, when the forests of the country were cleared. According to Dr. Willis's theory it must have been a much older inhabitant than, say, the indigenous endemic *Saccharum Ridleyi*, confined to the sandy heaths of Pahang. But we know that it is quite a modern introduction. Why should it be so much more abundant? The plant has an underground rhizome which can grow at a depth of 16 inches below ground, and if dug up can propagate itself rapidly from portions of the rhizome. Excessive heat and drought do not affect it, as the rhizome is too deep in the ground to be injured. Fire passing over the ground merely burns the leaves, which spring up again, growing an inch a day. The plumed seed is readily carried along into distant spots. I have even seen it growing in the sulphurous smoke of a volcano in Java, where hardly another plant could hold its own. We have another species in the country—*I. exaltata*, Brongn. This appears to have originated in South America, and occurs too in the New Hebrides and Malay islands, and the Malay Peninsula as far north as Mergui. It is closely allied, a rather taller

plant with the same plume-borne seeds. It was probably introduced into Singapore about the same time as the Lalang, but it has no deeply protected rhizome. It is unable to defend itself against great heat, drought, and fire. It occurs only sporadically here and there, and is in fact comparatively rare, and does not grow near volcanoes nor survive a forest fire.

Some years ago, in writing about cultivation of lawns, I had to point out that only Grasses with creeping rhizomes should be used, because in dry weather those which had not creeping rhizomes died out from the heat, while the rhizomatous Grasses creeping over each other prevented the ground from giving off the moisture (chiefly dew) all day during the heat.

Paspalum platycaule, Sw., is a creeping Grass, native of South America. It is now very common in Java. One day I found a patch of it in Singapore by the road-side, the only patch I had ever seen; in fact, it was VR. I removed some to the gardens, where it grew very fast; it now spread all over the Singapore road-sides. It has since become a nuisance, having become in a few years VC.

But the whole story of weeds (i.e. plants introduced by man) is the same. Look at *Ageratum conyzoides*, an American plant now extraordinarily common all over the Eastern tropics; *Clitoria cajanifolia*, formerly a rather rare local plant in Pernambuco, whence for many years only one or two specimens had been obtained, now common in Java and in Singapore, spreading to Borneo, thanks to the fact that, unlike other species of the genus, the seeds are viscid and adhere to the passing cattle; or again *Tithonia diversifolia*, Gray, a Mexican plant—'though only introduced (into Ceylon) as a garden plant so recently as 1851 it is now one of the commonest and most conspicuous plants in the island' (Trimen's Flora).

The obvious reason why wide range (as in the case of *Imperata cylindrica*) involves greater commonness is that for some reason the plant has advantages which enable it to spread and propagate its seed successfully and continuously, and has nothing to do with the period at which it entered the country.

What the advantages are in each plant is only to be known by careful study in the field, and an extremely interesting study it is, especially as they are different in almost every species, and due to modifications in all parts of the structure from root to seed, and even in some cases the chemical contents of the cell. Let the botanist find out why *Ageratum conyzoides* has spread over the whole tropics in spite of man, and *A. peruvianum* has failed though helped by man; why *Capsicum minimum* has established itself on tropical Eastern limestone rocks in immense abundance and *C. annum* failed.

These stories can be elucidated in the field, but not in the library.

SUMMARY.

Dr. Willis bases his arguments on the number of plants marked VC, VR in Trimen's 'Flora of Ceylon', and states that a VC plant cannot disappear without a geological catastrophe. It is shown that the rarity or commonness of plants in Ceylon, as based on this book, is unreliable for practical purposes, and that the VC species can and do disappear without any catastrophe.

Endemic species in Ceylon and elsewhere are nearly all the relics of an old flora rapidly disappearing, and in most cases cannot be evolutions of a later date, as there is nothing in the land from which they can have evolved, and therefore they must be the oldest, not the youngest part of the flora.

The mutation theory that new characters arise at a step, and that once they have appeared they remain hereditary and do not revert, is not in accordance with the facts. The theory fails to account for the adaptation of plants to their surroundings, and no theory yet proposed, except that of Natural Selection, does so.

The struggle for existence (more correctly the struggle for continuous reproduction) is not confined to the seedling, but is continuous throughout the life of the plant, and a mutation, however apparently trivial, of any organ in the plant may be adapted for this end.

It has been of course impossible in this paper to do more than give illustrative examples on any one point; the amount of evidence against the mutation theory and the various points controverted above is too enormous for even one book. This class of work can only be done in the field. Arguments based on calculations made from a book, however well written, are unreliable and misleading.

Dr. Trimen's Flora, on which Dr. Willis bases his arguments, is a good Flora for its date, but a great deal of research work has been since done on the countries adjacent to Ceylon, and from which the Ceylon flora took its origin. Most of the Ceylon species require critical examination again. There have been many misidentifications and errors in the geographical distribution, and very many of the species are but little known at present. With this work the study of the Ceylon flora commences, but the story is by no means complete yet, nor will it be till all the plants have been carefully and completely studied, one by one, in their relations to all conditions in which they occur in their natural wild state.

Seedling Anatomy of certain Amentiferae.

BY

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With eighteen Figures in the Text.

THE present paper deals with the seedling anatomy of those forms somewhat loosely classified as Amentiferae, an investigation of which was suggested by Dr. E. N. Thomas and carried out under her direction at Bedford College.

The group Amentiferae has here been regarded as consisting of the earlier cohorts of the Archichlamydeae of Engler up to and including the Urticales.

The only account so far published dealing with seedling anatomy in any of these cohorts is that of the Piperales by Mr. T. G. Hill.¹

The majority of the species to be described are members of the Juglandales and of the Fagales. Forms belonging to Verticillatae, Myricales, and Salicales have been obtained, but in the case of several of the smaller cohorts no material has been available.

Although in point of number of species representation is somewhat inadequate, the available forms in the larger cohorts Juglandales and Fagales cover a wide range as regards size of seed and habit of seedling. In addition to the difficulty of obtaining seeds the work has been much hindered on account of the long germination periods required by many of those which have been secured.

With the exception of a few individuals of British species collected in the field, most of the seedlings described have been raised at Bedford College.

The material has been examined chiefly by means of microtome series for which the best staining combination was found to be Bismarck brown and gentian violet. For hand sections a method of staining with Bismarck brown after treatment with sodium hypochlorite and acetic acid (recommended by Chauveaud² for early stages of phloem differentiation) has been found very useful.

¹ Hill, T. G.: Seedling Anatomy of the Piperales. *Ann. of Bot.*, vol. xx, 1906.

² Chauveaud, G.: Sur l'évolution des tubes criblés primaires. *Compt. rend. Acad. des Sc.*, t. cxxv, 1897.

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The chief aim of the investigation has been to record the earlier phases of primary differentiation of vascular tissue in the seedlings. To this end the material has been cut as far as possible in the youngest stage in which such differentiation could be demonstrated. Owing to differences in habit and rate of growth, correlated with differences in the rate of succession of the phases of vascular development, it is not possible to fix or exactly define any one stage which shall be equally applicable to all the seedlings.

The types of seedling include, on the one hand, slender epigeal forms in which the cotyledons are elevated above the soil by intercalary elongation of the hypocotyl, development of the plumular bud being meanwhile retarded (e.g. *Alnus*, *Carpinus*, &c.) ; and, on the other hand, massive hypogeal forms (e.g. species of *Fagians*, *Castanea*, &c.) in which the axis of the plumule may be more or less well developed in the seed. In these forms growth at first takes place almost simultaneously in both hypocotyl and epicotyl, but subsequently the latter elongates rapidly while the former remains very short.

In the first case there exists a well-defined stage during which the plumular strands are not sufficiently differentiated to exert any influence on the essential structure of the vascular system of the hypocotyl, and description of transition phenomena involves only the vascular strands in primary root, hypocotyl, and cotyledons.

In the second case there is no stage in which the plumular influence can be thus non-existent, since the earliest primary differentiation of vascular tissue will extend throughout the root, hypocotyl, and epicotyl. 'Transition' here includes the connexion of the vascular system of the root and of the hypocotyl with the early leaves, both cotyledonary and plumular.

In both cases cambial development may begin so early in the region of the cotyledonary node that primary differentiation is obscured, and in some cases can scarcely be said to exist.

The most striking anatomical feature of the forms under consideration is the absence of that uniformity of structure which has been found to be so pronounced in other groups. The variations which occur relate to number and position (these factors being to a great extent correlated) of the protoxylem poles in hypocotyl and root. All the modifications may be referred to Type 3 of Van Tieghem, in which transition from stem to root structure is brought about by 'movements' of the phloem strands while the xylem centres remain fixed.

In describing the various types of structure the terms *Cruciform* and *Diagonal* (see Fig. 1) will be applied in the sense in which they are used by Dr. Thomas¹ in her recent paper.

¹ Thomas, E. N. : Seedling Anatomy of Ranales, Rhocadales, and Rosales. *Ann. of Bot.*, vol. xxviii, 1914, p. 698.

It will be remembered that there exist two modifications of the Cruciform type: the *tetrarch* as described for *Althaea rosea* and many Rosales,¹ and as occurring also in Leguminosae,² Cactaceae,³ Tubiflorae,⁴ &c., and the *diarch* as described for Ranales and Rhoeadales, and for many other cohorts.⁵ In the former case four root poles are present, two in the cotyledonary and two in the intercotyledonary planes respectively, while in the latter case only the cotyledonary poles are present. Examples of both the above types have been found among the Amentiferae to be described.

In the Diagonal type the root poles occur in planes lying between the cotyledonary and intercotyledonary planes. The following diagonal modifications known to exist in other groups⁶ occur relatively frequently in the Amentiferae: diagonal tetrarchy (e. g. *Alnus cordifolia*), hexarchy, in which the preceding modification is combined with cruciform diarchy (e. g. *Quercus*, *Castanea*), octarchy, a combination of cruciform and diagonal tetrarchy (e. g. *Carya amara*), and in addition a double diagonal octarch type in which eight xylem centres occur on diagonals lying in pairs between the cotyledonary and intercotyledonary planes (Fig. 1, *a, b, c, d*).

This diagonal octarchy appears to be of constant occurrence only in *Fagus sylvatica*. It is shown by some individuals of *Quercus Ilex*, and has been described by Miss W. Smith⁷ in a seedling of *Palaquium petiolare*, a member of the Sapotaceae.

In the published accounts of other groups the greater number of forms are shown to possess one or other modification of the cruciform type, diarchy being by far the most common. The diagonal type is rare, but has been described in Calycanthaceae and in Rosaceae by Dr. Thomas, in Sapotaceae by Miss W. Smith,⁸ and in Ebenaceae by Mr. Wright.⁹

In the Amentiferous forms herein dealt with (exclusive of the Piperales and Urticales), diarchy is comparatively rare, since it is found only in the Salicales and in *Betula* and *Alnus* among Fagales. In the remaining forms cruciform tetrarchy and the various diagonal modifications occur with about equal frequency. Diarchy would seem to be the characteristic type.

¹ Thomas, E. N.: loc. cit., p. 698.

² Compton, R. H.: Seedling Structure in Leguminosae. Journ. Linn. Soc. Bot., vol. xli, 1912.

³ de Fraine, E.: Seedling Anatomy of certain Cactaceae. Ann. of Bot., vol. xxiv, 1910.

⁴ Lee, E.: Seedling Anatomy of certain Sympetalae. Pt. I. Ann. of Bot., vol. xxvi, 1912. Pt. II. Id., vol. xxviii, 1914.

⁵ Hill, T. G.: Piperales, loc. cit. Hill, T. G., and de Fraine, E.: Seedling Structure of certain Centrospermae. Ann. of Bot., vol. xxvi, 1912. Chauveaud, G.: L'Appareil conducteur des plantes vasculaires. Ann. Sci. Nat., Bot., 9^e sér., t. xlii, 1911.

⁶ Thomas, E. N.: loc. cit., p. 698.

⁷ Smith, W.: The Anatomy of some Sapotaceous Seedlings. Trans. Linn. Soc., ser. ii, Bot., vol. vii, 1909.

⁸ loc. cit.

⁹ Wright, H.: Genus *Diospyros* in Ceylon. Ann. Roy. Bot. Gard., Peradeniya, vol. ii, pt. 1, 1904, pp. 62-5.

for the Urticales and also for the Piperales described in the works of T. G. Hill¹ and of A. W. Hill.²

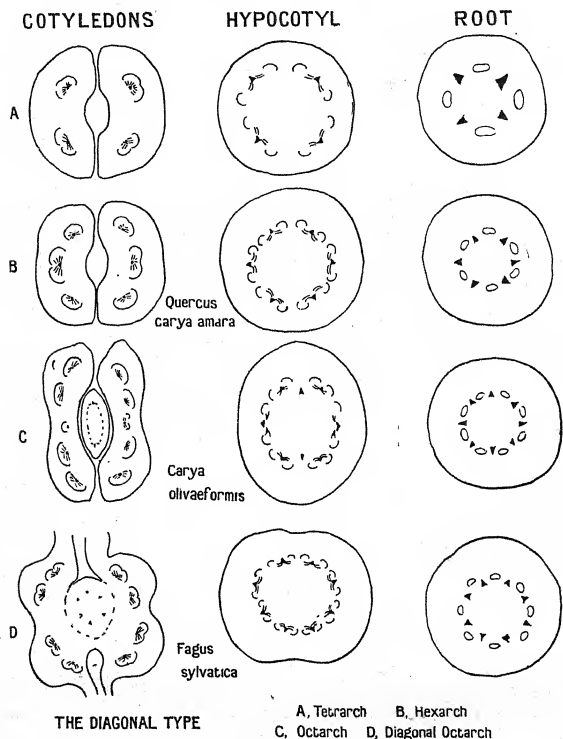


FIG. 1. Diagrams illustrating modifications of the diagonal type.

Since, however, so many of the earlier cohorts supply only a limited range as regards number of forms, comparison of this kind with the large groups described by other authors is of little value except perhaps in the case

¹ Hill, T. G.: loc. cit.

² Hill, A. W.: Ann. of Bot., vol. xx, 1906.

of the Fagales, a cohort of considerable size which presents instances of all the above-mentioned modifications.

The Amentiferae furnish interesting examples of the various degrees of connexion between the vascular system of the primary root and hypocotyl with the strands of the plumular leaves, similar to those which have been observed by Mr. Compton¹ in the Leguminosae. The traces of the early plumular leaves may show double bundle or triad structure to a marked degree.

VERTICILLATAE.

Casuarinaceae.

Casuarina. The species examined possessed slender, wiry seedlings; there are two epigeal cotyledons and a relatively long hypocotyl. The plumular axis develops earlier than is usual for this type of seedling. In the youngest individuals obtained, the minute bud enclosed within the short cotyledonary tube possessed three leaf whorls with well-differentiated vascular system. At the first plumular node there is a pair of rudimentary leaves alternating with the cotyledons, while successive nodes bear leaf sheaths, like those on the adult branches, consisting of four to six members. Well-developed buds which expand early are present in the axils of the cotyledons.

Casuarina equisetifolia. The cotyledons fuse into a short tube above the node. In the epicotyl each member of the leaf whorls contributes a single vascular strand. At the cotyledonary node six such strands are present, two in the intercotyledonary plane (from the first pair of leaves) and four diagonally placed (related to the members of the second whorl). These strands close up, forming two groups of three in the intercotyledonary plane. The cotyledons show an extended double bundle in which the medianly placed protoxylem becomes exarch at the node. Two lateral strands are present in the base of the petiole resulting from the union of more numerous ones at higher levels. The laterals sometimes approach the diverging halves of the central strand so that their phloem portions merge. The three strands of each cotyledon enter the axis without change in their relative positions. The central cotyledonary protoxylem continues downwards in the cotyledonary plane, while the metaxylem diverges from it. The metaxylem and protoxylem of the laterals approach the intercotyledonary plane, while elements of an earlier stage of differentiation appear in connexion with it on the sides adjacent to the poles. At a relatively high level in the hypocotyl the lateral metaxylems become united across the intercotyledonary plane, owing partly to contraction of the stele as a whole, and partly to the presence of centrally placed protoxylem which is not differentiated near the node. The phloem of the

¹ loc. cit.

plumular strands in the intercotyledonary plane becomes divided and united to the cotyledonary phloem in the diagonal corners. A few xylem elements from the plumular leaf-trace persist for a time external to the central intercotyledonary protoxylem. The hypocotyl stele now consists of four protoxylem poles, two in the cotyledonary and two in the intercotyledonary planes in connexion with tangentially differentiated metaxylem, and alternating with four phloem groups diagonally placed. An endodermis is present. In passing downwards the pith becomes smaller and tangential differentiation of the metaxylem is gradually replaced by centripetal, so that a typical tetrarch root structure is obtained above the collet.

In *C. tenuissima* and *C. stricta* the anatomical features are similar to those above described. In the latter species an older seedling showed that secondary growth begins very early in the region of the cotyledonary node, but is delayed at lower levels.

C. inophloia, described by Chauveaud,¹ is tetrarch and resembles the above species.

In these forms 'transition' takes place in accordance with the cruciform tetrarch type previously referred to (see p. 577).

PIPERALES.

Mr. T. G. Hill² has described the seedling anatomy of members of this cohort, including several species of *Piper* and of *Peperomia*, all of which appear to conform to the diarch type. *Peperomia peruviana*, a geophytic form described by Mr. A. W. Hill,³ and *Piper excelsum* referred to by M. Chauveaud⁴ are also diarch.

SALICALES.

Salicaceae. Seeds of several species of *Salix* and of *Populus* were obtained, but all except those which were sown immediately after ripening failed to germinate.

Salix caprea, *S. repens*. These have very small epigeal seedlings. In each cotyledon petiole there is an extended strand which is resolved into a double bundle at the node. The two double bundles together constitute in the hypocotyl a diarch stele having central protoxylem in the cotyledonary plane flanked by tangential metaxylem wings, and four phloem groups diagonally placed. Contraction of the stele with fusion of neighbouring phloems in the intercotyledonary plane produces a normal diarch root.

MYRICALES.

Myricaceae. Seedlings of only two species were obtained, and these were almost indistinguishable in external appearance. They are small, wiry, and slow growing. The cotyledons are epigeal. The first plumular

¹ loc. cit., p. 302.

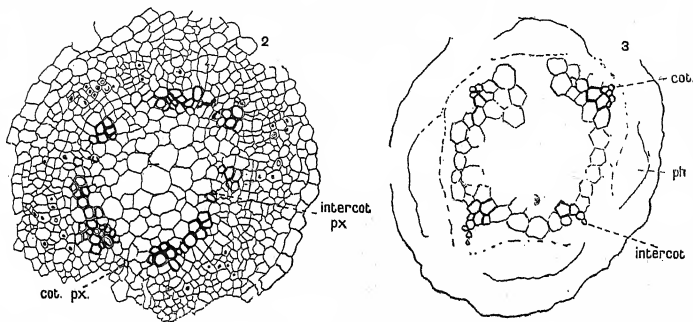
² loc. cit.

³ loc. cit.

⁴ loc. cit.

leaf is prominent at an early stage and causes slight asymmetry in the structure of the node.

Myrica californica (Fig. 18, III). The cotyledon petioles each possess a double bundle whose metaxylem and phloem diverge so as to be laterally placed with respect to the median protoxylem. At the node this protoxylem occupies the cotyledonary plane with metaxylem spreading tangentially on either side of it. In the upper part of the hypocotyl the stele conforms to the diarch type (see p. 577). The central protoxylems with their adjacent metaxylem wings constitute xylem arcs or secants in the cotyledonary plane, while the four phloem groups occupy the diagonal corners. In the intercotyledonary plane are the traces of the first and second plumular leaves, slightly differentiated as regards primary elements,

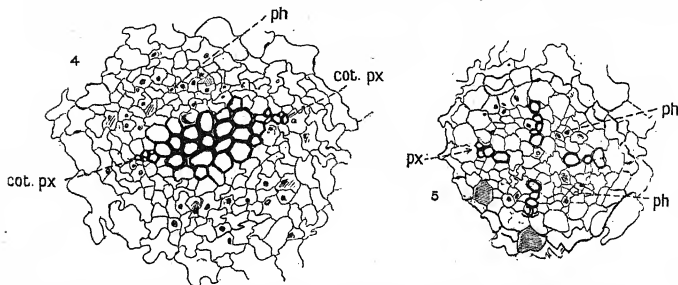


FIGS. 2 and 3. *Myrica californica*. 2. Transverse section of hypocotyl near cotyledonary node. $\times 280$. 3. Transverse section of root. $\times 320$. *ph.* = phloem; *cot.* = cotyledonary plane; *intercot.* = intercotyledonary plane; *px.* = protoxylem.

but linked with neighbouring cotyledonary traces by cambium which produces two bands of vascular tissue leaving wide gaps outside the protoxylem poles in the cotyledonary plane. In descending the hypocotyl this secondary tissue dies out. The phloem of the plumular trace persists for a time; it becomes divided, and its halves unite with the adjacent cotyledonary phloems. In the intercotyledonary plane feebly differentiated protoxylem elements appear, with metaxylem spreading tangentially on either side of them and increasing in amount as the hypocotyl is descended (Fig. 2). A tetrarch stele results, with protoxylems at the cotyledonary and intercotyledonary poles flanked by tangentially spreading metaxylems which eventually meet so as to form a lignified ring around the pith (Fig. 3). A well-differentiated endodermis is present. The stele

becomes more compact at lower levels, while lignification proceeds centripetally rather than tangentially from the protoxylem centres. In the region of the external collet the walls of the endodermis become much thickened. Tetrarchy persists throughout the root.

Myrica Gale. The structure of the cotyledonary traces and their behaviour on entering the axis resembles what is found in *M. californica*. At the node the cotyledonary and plumular strands unite to form a very compact stele in which the pith soon becomes obliterated by lignified elements. The cotyledonary phloems unite in the intercotyledonary plane in the manner of the diarch type. At a high level the stele shows the structure of a diarch root with a somewhat broad xylem plate, surrounded by a well-differentiated endodermis (Fig. 4). Diarchy extends



FIGS. 4 and 5. *Myrica Gale*. 4. Transverse section of lower part of hypocotyl, showing diarch root-structure. 5. Transverse section of root of same seedling near its apex. cot. px. = protoxylem of cotyledon; ph. = phloem. Both $\times 480$.

throughout the hypocotyl. In the region of the collet extensions of the xylem mass in the form of metaxylem and exarch protoxylem elements bisect each phloem group in the intercotyledonary plane. The cruciform tetrarch arrangement thus produced is continued downwards throughout the root (Fig. 5).

The above species agree in the possession of a tetrarch root, and also in the diarch structure of their hypocotyls near the cotyledonary node. In *M. Gale* the intercotyledonary protoxylem poles do not extend upwards above the collet, and the hypocotyl structure is that of a diarch root, but in *M. californica* four phloem groups always remain distinct, and the intercotyledonary protoxylem poles extend upwards through the greater part of the hypocotyl.

JUGLANDALES.

Juglandaceae. This family includes both hypogeal and epigeal forms, in all of which the cotyledons are deeply bifurcated, the resulting halves being again divided.

All the species of *Juglans* and *Carya* examined possess large seeds and robust seedlings. The bifurcation of the massive folded cotyledons extends down to where the lamina merges into a broad flattened petiolar region. There is a very short thick hypocotyl and a stout primary root. The plumular axis lengthens at an early stage and bears scales like rudimentary leaves which show gradual transitions to the mature form.

Juglans nigra. The cotyledon petioles each contain a small central bundle flanked by two pairs of large lateral strands which supply the four lobes of the lamina. These lateral strands are extended and somewhat incurved, and smaller branches from the lamina sometimes unite with

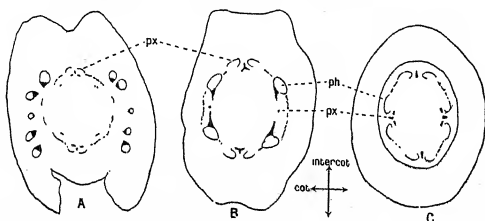


FIG. 6. *Juglans nigra*. A. Cotyledonary node. B. Hypocotyl. C. Root. *px.* = protoxylem; *ph.* = phloem. Arrows show direction of cotyledonary and intercotyledonary planes.

them in such a position as to produce inverse orientation. This does not extend into the node.

The central cotyledonary trace enters the axis in the cotyledonary plane and is resolved into a double bundle. The adjacent laterals approach each other and enter as a more or less compact group in the diagonal planes. The intercotyledonary poles are occupied by the traces of the first two plumular leaves. Each consists of a central protoxylem group flanked by metaxylem, beyond which are two divergent phloem groups (Fig. 6). The structure is that of the triad unit considered by Dr. Thomas¹ to be characteristic of cotyledonary and probably also of plumular traces.

The triad arrangement is continued upwards in the epicotyl until the exit of the leaf-traces as double bundles with loss of their central protoxylem. In the short hypocotyl, root poles are organized in the cotyle-

¹ loc. cit., p. 732, and Text-fig. 28, p. 716 (*Draba Aisoon*).

donary plane from the central strands of the cotyledons, their metaxylems being supplemented by that of the lateral groups. Widely extended tangential masses of metaxylem result, as the stele surrounds a very large pith. In the intercotyledonary plane the amount of metaxylem helping to constitute the plumular traces becomes increased, and they continue downwards as root poles with which the cotyledonary strands make no connexion (Fig. 6, A, B, C). In the cruciform tetrarch root all the poles are equivalent, and the differentiation of their primary xylem becomes entirely centripetal. Groups of plumular metaxylem in the diagonal planes also extend into the root, and neighbouring cotyledonary phloem groups do not

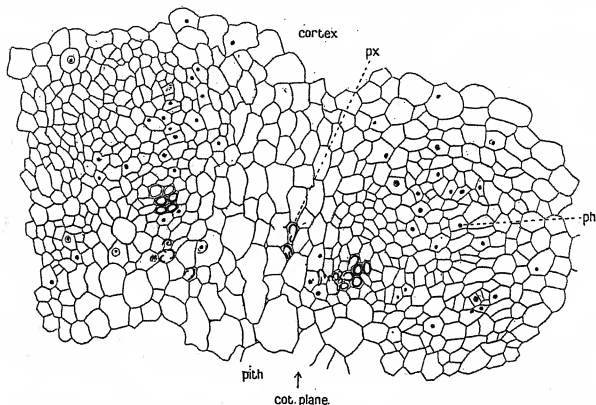


FIG. 7. *Juglans nigra*. Part of transverse section of very young epicotyl, showing one of the plumular leaf-traces in cotyledonary plane (*cot. plane*). *px.* = protoxylem; *ph.* = phloem. $\times 360$.

appear to meet, perhaps owing to the large size of the stelar ring, but become linked by phloem developed later in connexion with plumular traces. The hypocotyl stele is surrounded by a well-marked endodermal sheath, portions of which leave the axis with the cotyledon traces. Within the endodermis there is a wide pericycle consisting of several layers of cells. Secondary thickening takes place at an early stage.

In this seedling there has been traced the complete connexion of a plumular leaf with an intercotyledonary root pole by means of a vascular strand showing uniform structure in hypocotyl and epicotyl. Similar connexions have been described by Compton¹ as occurring in *Caesalpinia sepiaria* and in *Pithecolobium Unguis-cati*, members of the Leguminosae.

¹ *loc. cit.*, pp. 9 and 21.

Fuglans Sieboldiana. This seedling agrees with the preceding species in the structure of its cotyledon strands and in their arrangement at the cotyledonary node. The traces of the first and second plumular leaves in the intercotyledonary plane show double structure with isolated median protoxylem, but this last feature is not continued into the epicotyl, where the leaf-trace consists of two widely separated endarch bundles. The structure of the hypocotyl and of the root is tetrarch and resembles that of the last species, but some of the xylem of the lateral cotyledonary strands diverges towards the intercotyledonary plane, where it becomes connected with the plumular strands. The greater part of the metaxylem of the intercotyledonary root poles is continuous with that of the cotyledonary strands, but the central portions are continued up into the epicotyl as the traces

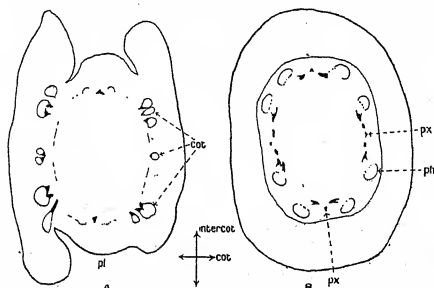


FIG. 8. *Fuglans Sieboldiana*. Outlines of vascular system. A. At cotyledonary node. B. At a lower level in the hypocotyl. cot. = cotyledonary traces. Arrows indicate cotyledonary and intercotyledonary planes.

of the first two leaves. In other respects, such as the presence of a large pith and early secondary thickening, and also in the fact that metaxylem from later plumular leaves may persist in the diagonal planes, the seedling is similar to that of *Fuglans nigra*.

Fuglans cinerea. The cotyledon petioles show the usual five strands, but the central one is extremely small, and the constituents of the laterals are not compactly united. These enter the axis as groups of two or three approximated bundles at the diagonal corners. The plumular strands are very slightly differentiated, but show doubleness of their phloem. In the hypocotyl, the cotyledonary poles are supplied by the central strands together with adjacent parts of the lateral groups, the remaining portions of which are connected with the intercotyledonary poles as in the normal tetrarch type. No plumular tissue takes part in the primary organization of root poles.

Fuglans regia. This seedling showed tetrarchy in root and hypocotyl

related to central and lateral cotyledonary traces as in *Juglans cinerea*. The material available was so old that secondary thickening in the region of the cotyledonary node prevented the primary structure of the plumular traces from being followed with certainty.

The genus *Juglans* is interesting, since in some species there is complete continuity of a vascular unit similarly constituted throughout root, hypocotyl, and epicotyl. In all the species there is great similarity in the structure of the hypocotyledonary stele.

The seeds and seedlings of the genus *Carya* are not so large as those of the species of *Juglans* described above, but in habit and mode of germination they are similar.

Carya olivaeformis. This seedling is smaller and slenderer than that of the other species described. The hypocotyl possesses eight protoxylem centres surrounded by the customary tangential metaxylem and diverging phloem groups. Passing upwards, six of the xylem poles, four diagonal and two cotyledonary, are continuous with the xylem of the central and lateral cotyledon traces respectively. The two remaining intercotyledonary strands with their related phloems extend up into the epicotyl, forming the traces of the first two plumular leaves exactly as in *Juglans nigra*.

In young seedlings octarchy persists throughout the root, but in one somewhat older individual the intercotyledonary poles diminished in size and became merged with one of the neighbouring diagonal ones by suppression of the intervening phloem group. The cotyledonary poles became less well developed, and were represented at low levels by tangential arcs of xylem. Rootlets arose in connexion with the diagonal poles only.

This seedling shows the combination of diagonal and cruciform tetrarchy, and is further interesting in that it possesses well-developed intercotyledonary root poles organized in connexion with plumular leaf-traces as in *Juglans nigra*.

Carya amara. The only seedling obtained was very old and had produced great length of root with much secondary thickening, although the plumule had not emerged from the seed.

In the hexarch root two of the poles are in the cotyledonary plane, while the remaining four are diagonal. The poles persist in the hypocotyl and are connected with the central and two lateral double bundles in the cotyledon bases. Higher in the cotyledon petiole there are six bundles arranged in pairs, each being surrounded by a single layered sheath of cells. A thick-walled tannin containing endodermis surrounds the stele in both root and hypocotyl. At the cotyledonary node portions of it pass out with the cotyledon traces and are continuous with the bundle sheath in the petioles.

Pterocarya rhoifolia (Fig. 18, VI). This species has a fairly robust epigeal seedling with a long hypocotyl. The cotyledons (deeply four-lobed as in the other genera) are thin and leaflike. Their petioles possess two pairs of lateral strands and a small central one as in *Fuglans*. Near the node the neighbouring lateral strands approach and fuse, while the central strand becomes divided, its halves merging with the laterals. There thus result two large divergent strands which enter the axis at diagonal corners as endarch bundles. At this level there is no central protoxylem. The first plumular leaf-trace shows slight lignification which dies out at the cotyledonary node. The cotyledonary metaxylems become divided, and their halves approach the neighbouring cotyledonary and intercotyledonary planes, where at lower levels protoxylem is differentiated slightly external to the metaxylem. Protoxylem and metaxylem become approximated so as to produce flattened secants about the cotyledonary and intercotyledonary poles. At this stage the stele is very large, and the diagonally placed phloems form much-extended tangential bands. In the region of the collet only centripetal development of the xylem occurs. The root is tetrarch and shows a very large pith. A thin-walled endodermis with the characteristic radial dot is present in the greater part of the hypocotyl.

Fortunea chinensis (Fig. 18). This seedling, although smaller than that of *Pterocarya*, resembles it closely in habit and also in anatomical structure, except for the fact that the central cotyledonary protoxylem of the hypocotyl extends upwards into the minute central strand of the cotyledon.

FAGALES.

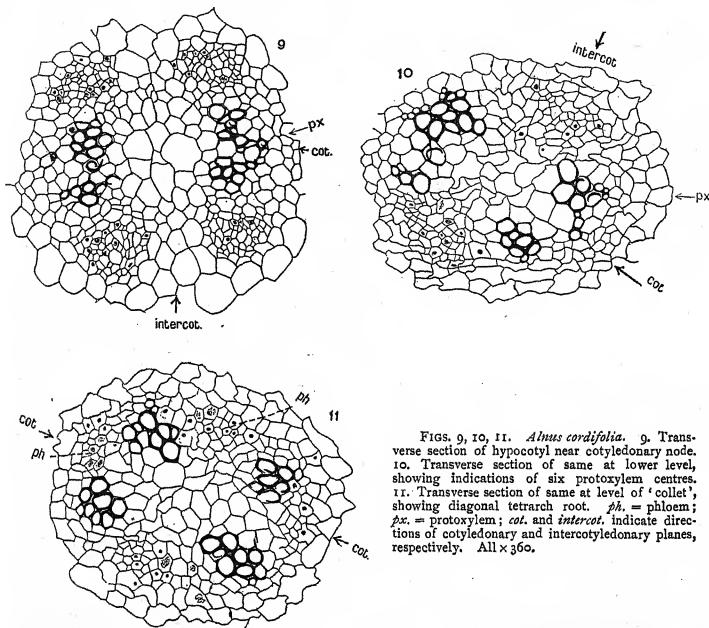
Betulaceae. With the exception of *Corylus Avellana* the seedlings of this family are small and slender, having an elongated hypocotyl and epigeal cotyledons. Portions of the cotyledon blades are prolonged downwards as auricles.

Alnus incana, *A. glutinosa*, and *A. cordifolia*. These are slender, wiry seedlings resembling each other very closely. In all three species the cotyledon petiole shows a double bundle, the halves of which diverge widely. The cotyledon traces enter the hypocotyl with isolated central protoxylem in the cotyledonary plane flanked by metaxylem groups, beyond which are the phloems in the diagonal positions.

In *Alnus incana* and *A. glutinosa* normal diarch structure is attained in the hypocotyl by union of neighbouring phloem groups in the intercotyledonary plane together with compacting of the xylem secants in the cotyledonary plane. In *A. glutinosa* the phloem groups remain apart for a longer period than in *A. incana*. In both species the root is diarch.

In *Alnus cordifolia* (Fig. 9) the structure of the node and of the upper part of the hypocotyl resembles that found in the species above.

described, but the central cotyledonary protoxylem may be feebly developed. Neighbouring phloems unite in the intercotyledonary plane at a high level. In descending the hypocotyl the central cotyledonary protoxylem gradually dies out, while the metaxylem becomes extended, and exarch protoxylem groups appear in contact with it at the diagonal corners



FIGS. 9, 10, 11. *Alnus cordifolia*. 9. Transverse section of hypocotyl near cotyledonary node. 10. Transverse section of same at lower level, showing indications of six protoxylem centres. 11. Transverse section of same at level of 'collet', showing diagonal tetrarch root. *ph.* = phloem; *px.* = protoxylem; *cot.* and *intercot.* indicate directions of cotyledonary and intercotyledonary planes, respectively. All $\times 360$.

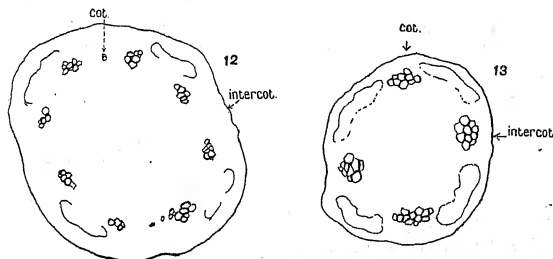
of the stele (Fig. 9). For a time six protoxylem centres may be present, but the original cotyledonary groups cease to be differentiated, while those diagonally placed persist into the root. In the region marked by the external collet, phloem is differentiated in the cotyledonary plane; thus completing a tetrarch root stele (Fig. 11). This method of formation of a diagonal tetrarch root recalls what is described by Chauveaud¹

¹ loc. cit., p. 320.

for *Calycanthus occidentalis*, in which the phloem strands present in the cotyledonary plane of the root die out in the base of the hypocotyl.

Betula excelsa and *B. pumila*. These are very small seedlings, resembling in habit those of *Alnus*. In both species the root is diarch and the transition features are precisely similar to those of *Alnus incana*.

The following features occur in all the above species of *Alnus* and *Betula*. The first plumular leaf is prominent, and vascular traces from this and the succeeding leaf are present at the cotyledonary node in the intercotyledonary plane. These consist almost entirely of secondary tissue and do not extend far into the hypocotyl. At an early stage the cambium develops so as to link the cotyledonary and plumular traces together, producing two bands of vascular tissue extending at right angles to the



FIGS. 12 and 13. *Carpinus Betulus*. 12. Vascular system of hypocotyl showing protoxylem in cotyledonary plane. 13. Root of same. cot. and intercot. indicate the respective planes.

cotyledonary plane, as in *Myrica*. An endodermis showing the characteristic radial dot makes its appearance at a high level in the hypocotyl.

Carpinus Betulus and *C. Ostrya*. These seedlings are larger than those of *Alnus*. The cotyledons are slightly fleshy and remain within the seed longer than do those of the preceding genera.

The anatomical features of both species are exactly similar. The cotyledon petioles possess each a large double bundle with widely diverging halves which enter the node at the diagonal corners as collateral endarch strands. Centrally placed protoxylem is present in the base of the petiole and at the cotyledonary node, but dies out immediately below it. In descending the hypocotyl each of the four metaxylem strands becomes divided into two by the failure to continue differentiation of its median elements (Fig. 12). The eight groups of metaxylem thus produced gradually 'approach' the neighbouring cotyledonary or intercotyledonary planes by means of the appearance of earlier lignified elements on the side

adjacent to the plane and the dying out of those remote from it. The stele becomes contracted and an endodermis is differentiated (Fig. 13). The central cotyledonary protoxylem reappears in the form of a few feebly lignified disorganizing elements. At lower levels similar protoxylem appears at the intercotyledonary poles. Near the collet the metaxylem makes contact with the protoxylem poles so that four somewhat extended xylem secants are formed, alternating with the phloem groups which have maintained their diagonal position. Below the collet the metaxylem becomes compacted and a tetrarch root stele results.

The first plumular leaf-trace is double at the cotyledonary node, but is differentiated so slightly before secondary thickening ensues that it makes no connexion with the primary structure of the hypocotyl. The cambium at first develops in two bands, as in *Alnus* and *Betula*.

Corylus Avellana. This is a large seedling with fleshy hypogeal cotyledons and a very short hypocotyl as externally limited. There is early elongation on the part of the plumular axis, which bears rudimentary leaves, as in *Fuglans* and *Carya*.

The cotyledon petioles each contain two massive and rather widely separated strands which enter the axis diagonally, as in *Carpinus*. At the cotyledonary node a small group of protoxylem is present between these strands, and can be traced downwards in the cotyledonary plane throughout the hypocotyl. The four groups of metaxylem from the cotyledons become each divided into two, and the resulting portions approach the neighbouring cotyledonary or intercotyledonary planes. Root poles are organized in the cotyledonary plane, as in *Carpinus*. In the intercotyledonary plane there persist the traces of the first and second plumular leaves. Their phloem in each case consists of two groups which diverge and unite with that of the cotyledons, while their xylem forms arcs of small scattered elements linking the adjacent cotyledonary metaxylems. Extended xylem secants consisting of plumular and cotyledonary elements are thus formed, and become compacted at lower levels into the intercotyledonary xylem poles of the cruciform tetrarch root. The replacement of tangential by centripetal development of the xylem takes place so gradually that typical root-structure is attained only at some distance below the collet.

In this seedling the intercotyledonary root poles are connected partly with the cotyledon traces and partly with those of plumular leaves, thus recalling *Fuglans Sieboldiana*. The plumular portion is not so well marked as in the latter species, and is continued into the epicotyl as a double strand without central protoxylem.

Fagaceae. With the exception of *Fagus sylvatica*, the members of this family to be described possess large hypogeal seedlings in which the early plumular leaves are rudimentary. In all the species the cotyledon blades are prolonged downwards below their junction with the petioles,

In the species of *Quercus* examined, the vascular structure of the cotyledonary node and hypocotyl conforms to the hexarch type (Fig. 1, B). The cotyledon petioles contain three massive vascular strands, of which the central one is double near the cotyledonary node, while the laterals are usually not obviously so until they enter the axis. Here the usual triad grouping takes place in connexion with all the strands. The hypocotyl contains six protoxylem centres (two cotyledonary and four diagonal), each flanked by scattered tangentially placed metaxylem. The adjacent phloems of neighbouring triads approach each other. The pith is very large. Plumular traces are present in the cotyledonary node and for a short distance below it, interpolated between the cotyledon strands.

Quercus robur. The hypocotyl and root show hexarch structure related to three strands in each cotyledon.

In one seedling the central strand was very small in one cotyledon and was entirely absent from the other. Five triads were organized in the hypocotyl for a short time, but only the four diagonal ones persisted, and the root was tetrarch.

Another individual showed extreme development of incurved traces in the cotyledon petioles. Irregularly grouped strands from the lamina came together, forming a much-extended central band with incurved ends and a pair of lateral strands in the form of more or less complete rings. Near the node the rings opened out and became organized as double bundles. Central protoxylem was not present above the node. The central band slowly became more compact and was resolved into a double bundle in which central protoxylem elements were present. These bands and rings consisted mainly of secondary tissue, the primary elements being very small and fugitive.

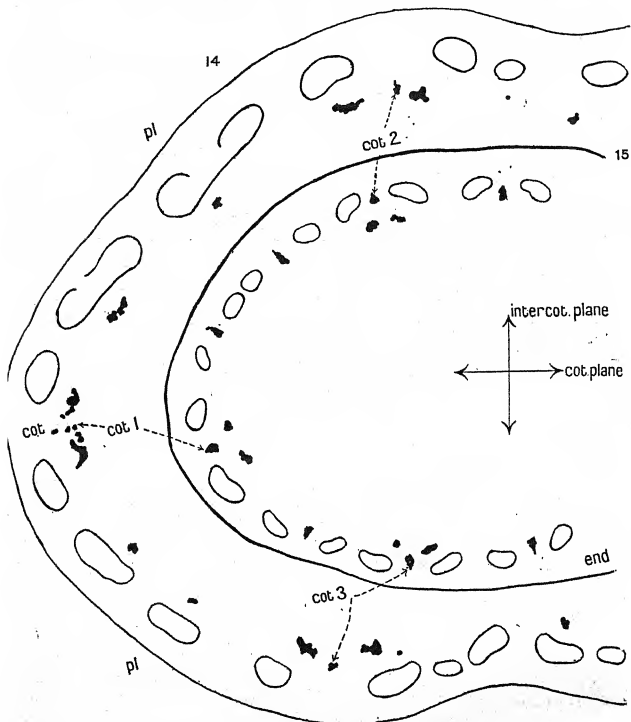
Quercus Ilex. The seeds and seedlings are somewhat smaller than those of the other species described.

In general the seedlings were hexarch, but the following modifications occurred. In some individuals, eight triads were present in the hypocotyl. These were arranged as in the double diagonal type. Each triad was continuous with one of four strands in each cotyledon, and occurred as laterally placed pairs; sometimes, however, the two innermost groups came together to form a single large central strand. A very small central strand was sometimes produced by the union of portions detached from the laterals. The root was diagonally octarch, as in *Fagus sylvatica*.

In a few otherwise normal seedlings there was increase in the number of root poles from six to seven or eight. This always took place at some distance below the collet and was observed only in relatively old seedlings.

Quercus Cerris. All the individuals examined followed the general hexarch type and showed no irregularities.

Castanea sativa. The seed and seedling are larger and more robust than is the case in *Quercus*; except in one instance, where four seeds had

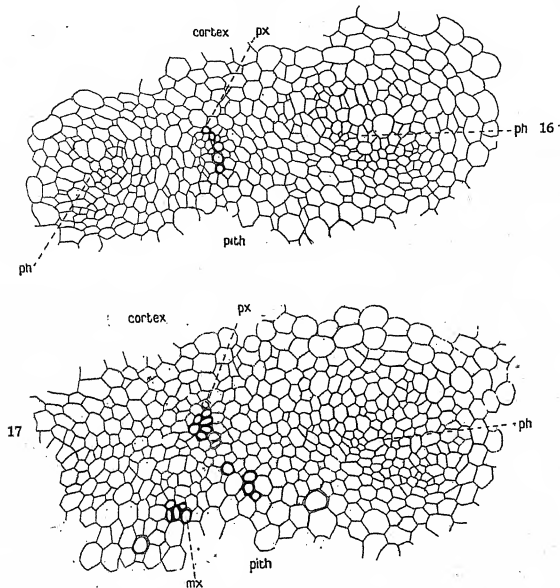


FIGS. 14 and 15. *Castanea sativa*. 14 (outer figure). Half of vascular system of hypocotyl just below cotyledonary node, showing cotyledonary (*cot. 1, cot. 2, &c.*) and plumular traces (*pl*). 15 (inner figure). Vascular system of the same seedling at a lower level, showing 'triads' of xylem and phloem corresponding to cotyledonary and plumular traces. The boundary line of each figure indicates the position of the endodermis. (Xylem, black; phloem, white.)

matured within a single fruit and all germinated. Their structure was similar to that of the larger seedlings.

The cotyledonary petioles possess three large vascular strands which

may be incurved, the laterals occasionally forming almost complete rings. The cotyledonary traces enter the hypocotyl and are continued downwards as triad units in accordance with the hexarch type described for *Quercus*. At the cotyledonary node, plumular leaf-traces in the form of slightly differentiated bundles or desmogen strands are interpolated between the



FIGS. 16 and 17. *Castanea sativa*. Details of vascular units indicated in Fig. 15. 16. A 'plumular' unit. 17. A cotyledonary unit (only one of the constituent phloem groups is shown at *ph.*). *px.*=protoxylem; *mx.*=metaxylem. Both $\times 360$.

cotyledon traces (Fig. 14). In descending the hypocotyl there is increase in the differentiation of the xylem elements of any pair of adjacent plumular strands, while an isolated protoxylem group appears between them. The vascular system of the hypocotyl thus comes to consist of a variable number of triad units, of which only six are related to the cotyledon traces (Fig. 14). The number of additional units may vary from two to eight, but is commonly six. At lower levels the lateral or tangential metaxylem of each triad fails

to be differentiated, while centripetal development proceeds from the central group. The lateral metaxylem of the cotyledonary triads persists downwards to a lower level than does that of the 'plumular' triads (Figs. 14, 15, and 16). Neighbouring phloems are slow in coming together, but in most cases a root stele was attained, showing from eight to fourteen equivalent protoxylem poles alternating with phloem groups. A wide pericycle and a well-defined endodermis are present in the root, and are continued up into the hypocotyl. In the youngest seedlings no root stage was observed, since the triad units persisted downwards independently as far as differentiation could be demonstrated.

It seems clear that the additional units of the hypocotyl and of the root are organized in continuity with the plumular strands found at higher levels; but the feebleness of differentiation in the upper regions of young seedlings, which is rapidly succeeded by cambial development in older stages, renders their exact connexion difficult of observation.

Fagus sylvatica. This is a large seedling of the epigeal type. The cotyledons, which are fleshy and much folded, are slow in expanding, and the elongation of the plumular axis is much retarded. All the plumular leaves are of the normal foliage type, the first two being opposite. The cotyledon petioles contain four lateral strands approximated in lateral pairs. These diverge to form the leading veins of the broad fan-shaped lamina. There is no indication of the central strand either in petiole or lamina. The vascular supply recalls that of the bifurcated cotyledons of Juglandaceae. At the base of each cotyledon petiole the four vascular strands are organized as double bundles. Eight of these enter the hypocotyl in diagonal planes, producing a symmetrical octarch structure. Metaxylem and phloem diverge more widely from the central protoxylem, which gradually assumes the exarch position. Throughout the long hypocotyl and for some distance below the collet the stele consists of eight triad groups; eventually the neighbouring phloems unite and an octarch root results. A large pith extends throughout hypocotyl and root.

The cambium, which begins its development in the upper part of the hypocotyl, is at first active only within the limits of each triad. Metaxylem and phloem become linked by secondary tissue, while the protoxylem disorganizes; so that the stele appears to consist of eight endarch bundles. A well-marked endodermis is present in the root and extends upwards throughout the greater part of the hypocotyl. A large number of seedlings examined at different stages failed to reveal any variations or irregularities such as were found in *Quercus*.

The seedlings of Fagales are of some interest, because they include examples of most of the known types of seedling anatomy together with forms showing transition from one to the other. Diarchy occurs in *Betula* and in certain species of *Alnus*. *Alnus cordifolia* shows diarchy

in the upper part of its hypocotyl replaced by diagonal tetrarchy in the root, a transitory hexarch stage being suggested in the intermediate region. Cruciform tetrarchy is found in *Carpinus* and in *Corylus*. In the latter seedling the intercotyledonary poles are connected in part with plumular strands, but not to so marked an extent as in certain Juglandaceae.

Hexarchy is the fundamental arrangement in the hypocotyl of *Quercus* and *Castanea*, but in the latter genus additional units are present which are probably related to plumular leaf-traces.

Throughout the group the early plumular leaf-traces show double structure in both epicotyl and hypocotyl. In the epigeal forms this usually applies only to the first pair of leaves, and in their case secondary thickening ensues so early that very little primary tissue is differentiated. In the hypogeal forms the growth of the plumule is so rapid that the traces of several leaves may be present at the cotyledonary node. These may extend into the hypocotyl and root, bearing the same relation to root poles as the cotyledonary double bundles.

URTICALES.

In this cohort only a few forms have been examined. All of them possess epigeal seedlings which differ considerably in size. Their anatomical features show striking uniformity.

Ulmaceae.

Celtis australis and *C. occidentalis*. These are moderately robust seedlings. The cotyledons possess a central double bundle, and transition is in accordance with the diarch type.

Moraceae.

Morus alba. The seedling is small and slender. The hypocotyl and root are diarch, transition taking place at a high level.

Maclura aurantiaca possesses a much more robust seedling, resembling those of the species of *Celtis*. Transition follows the diarch type.

Urticaceae.

Urtica cannabina has a small diarch seedling very similar to that of *Morus alba*.

Urtica dioica, *Parietaria officinalis*, and *Humulus Lupulus*, described by Chauveaud,¹ all conform to the diarch type.

General Remarks.

Speaking of the vascular structure of the seedlings of Ranales, Rosales, and Rhoeadales, Dr. Thomas² says that 'the hypocotyl shows a varying number of primary centres of xylem which alternate with two phloem

¹ loc. cit., p. 295.

² loc. cit., p. 732.

groups and from which differentiation proceeds in a more or less tangential direction on either side'. This statement may be applied equally to the Amentiferae at present under consideration, the structure of which, therefore, lends support to the view that the unit of vascular structure in the hypocotyl is the triad unit as above defined. The triads are continued upwards into the cotyledonary strands (and sometimes into plumular leaf-traces), where they constitute the familiar double bundles of so many authors.

In the Amentiferae, examples of the double bundle or triad structure in the traces of earlier plumular leaves are frequent (*Fuglans*, *Corylus*, &c.). At their base in the region of the cotyledonary node these traces usually die out or become merged with cotyledonary strands in the hypocotyl. In certain cases, notably *Fuglans nigra* and *Carya olivaeformis*, the triads of the first and second plumular leaves extend downwards into the hypocotyl as independent strands related to root poles in exactly the same way as the cotyledon traces.

In *Castanea sativa* there are interpolated between the cotyledonary traces a number of additional strands (usually seven or eight), which are well differentiated as triad units in hypocotyl and root, and correspond in position to the plumular leaf-traces or desmogen strands found at the cotyledonary node. It seems that there is continuity of unit strands between hypocotyl and plumular leaf. This would indicate that a large number of units in root and hypocotyl may be differentiated in connexion with rapidly developing plumular leaves. The number of leaves is not so great as might appear, since the three strands which enter the node from any one leaf may remain as independent centres.

In the genus *Fuglans* there are shown varying degrees of relationship of two hypocotyl units with the strands of the first and second plumular leaves. In *Fuglans nigra* the two intercotyledonary poles are in continuity with the triad units of plumular leaves. In *F. Sieboldiana* the central part of the strand is plumular, while the lateral portions diverge into the cotyledons; in *F. cinerea* the poles are entirely cotyledonary.

It is possible that a similar series might be established in the genus *Carya*, in which, so far, only two species have been examined: *C. olivaeformis*, which is octarch with its intercotyledonary poles entirely plumular, and *C. amara*, which is hexarch owing to the absence of poles in this position.

Similar phenomena¹ have been described by Compton² as occurring in members of the Leguminosae. Independent plumular intercotyledonary poles like those of the above-mentioned species of *Fuglans* and *Carya* have been found by him in *Pithecolobium Unguis-cati* and in *Caesalpinia sepiaria*.

¹ It is possible that the 'accessory' root bundles described by Miss W. Smith in *Bumelia tenax* (loc. cit., p. 192) may bear the same interpretation.

² loc. cit., pp. 9 and 21.

Of the smaller epigeal seedlings many are tetrarch, but very often the four poles are well differentiated only at low levels in the hypocotyl near the collet. The most extreme instance of this is furnished by *Myrica Gale* (see p. 582). It occurs also in *Carpinus* and *Alnus*. The intercotyledonary protoxylem poles persist upwards to a varying degree, but die out near the cotyledonary node; while the metaxylem diverges and passes out as part of

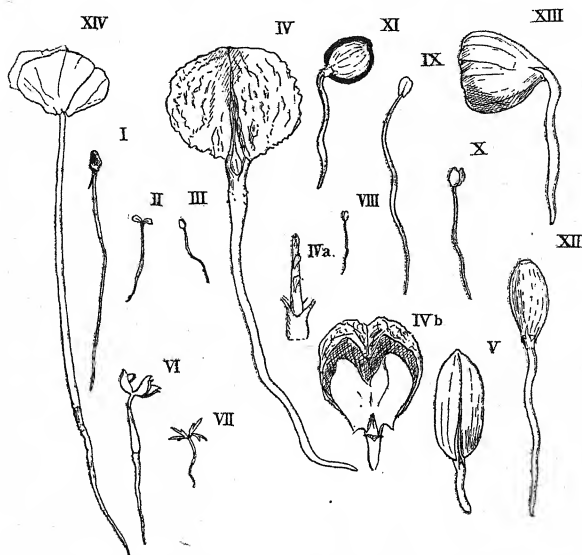


FIG. 18. Seedlings of Amentiferae (all figs. $\frac{2}{3}$ natural size). I. *Casuarina equisetifolia*. II. *Salix caprea*. III. *Myrica californica*. IV. *Juglans nigra* (IV a. plumule of same, IV b. younger seedling). V. *Carya olivaceaformis*. VI. *Pterocarya rhoifolia*. VII. *Fortunea chinensis*. VIII. *Betula pumila*. IX. *Carpinus Betulus*. X. *Alnus cordifolia*. XI. *Corylus Avellana*. XII. *Quercus Ilex*. XIII. *Caslanea sativa*. XIV. *Fagus sylvatica*.

the cotyledonary traces. In some cases the whole of the triad strand is divided between the cotyledons.

It is probable that here also the intercotyledonary centres are differentiated in relation to the plumular traces, which are frequently double strands. Since the plumular development is so much delayed, differentiation is not complete at the upper end of the seedling, so that the protoxylem

centre 'dies out' in ascending the hypocotyl, and actual connexion cannot be demonstrated.

Similar distribution of the intercotyledonary vascular units between cotyledons and plumular leaf has been recorded as obtaining in many Leguminosae by Compton, who describes it as a phenomenon of replacement in which the cotyledons are being supplanted by plumular leaves.

The variation of the level to which the intercotyledonary poles persist upwards is correlated with the rate at which the successive phases of vascular differentiation supersede one another as the hypocotyl is ascended.

The succession of phases which has been conclusively demonstrated by Chauveaud, and receives the support of Compton and of Thomas, accounts also for the discontinuity which sometimes exists between the central protoxylem in the base of the cotyledons and that present at lower levels. In the youngest stages, development of the xylem proceeds centripetally from the first centres of differentiation ('éléments alternés' of Chauveaud). This is followed by development in a tangential or lateral direction on either side of the centre, and this in turn by centrifugal differentiation, both primary and secondary.

As the seedling elongates, successive increments of growth will have their vascular differentiation initiated in continuity with the phase which is active in the preceding increment. In the hypocotyl the earliest differentiation takes place in the region of the collet and of the cotyledonary node. When there is much intercalary elongation between these two regions a succession of phases may be found in ascending from the collet and to a less extent in descending from the node, and thus there will be lack of continuity between, for example, the 'alterne' elements or central protoxylem of the cotyledonary petiole and those of the root pole (e.g. in *Carpinus*).

Although, in general, the massive hypogeal seedlings possess the larger number of root poles, there are very striking instances of the absence of a definite relation between size or habit and the number of root poles present. Thus tetrarchy is equally characteristic of the largest hypogeal seedlings examined (species of *Juglans*) and of the slender epigeal seedlings of *Casuarina* (as instanced by Thomas¹), *Myrica*, *Alnus cordifolia*, *Carpinus*, &c. The relatively slender epigeal seedling of *Fagus sylvatica*, while resembling in external habit the diarch and tetrarch species of *Calycanthus* described by Chauveaud and by Thomas, nevertheless exhibits diagonal octarchy.

¹ Thomas, E. N.: A Theory of the Double Leaf-trace founded on Seedling Structure. New Phyt., 1907, p. 85.

SUMMARY.

1. Members of the following cohorts have been examined: Verticillatae, Salicales, Myricales, Juglandales, Fagales, and Urticales.

2. Diagonal types of transition are of frequent occurrence, and are correlated with the presence of large numbers of root poles and hypocotyl strands.

3. Diarchy is characteristic of the Urticales and also of the Piperales described by Mr. T. G. Hill, but is otherwise seldom met with.

4. The form of the vascular strands in the hypocotyl is remarkably constant, and is that of the triad defined by Dr. Thomas as the unit of hypocotyl vascular structure.

5. The hypocotyl may contain triad units, in addition to those continuous with the double bundles of the cotyledons, which are related in a similar manner to plumular leaf-traces.

6. Doubleness of plumular leaf-traces occurs very generally.

In conclusion, I wish to thank Dr. Thomas for much helpful criticism and advice, and also* for having placed at my disposal for purposes of comparison her own preparations of the seedlings of other groups.



Some Points in the Morphology of the Stipules in the
Stellatae, with special reference to *Galium*.¹
(Additional Note.)

BY

H. TAKEDA, D.I.C.

With seven Figures in the Text.

THE writer, after his return to Japan, has had an opportunity of examining specimens of Stellatae preserved in the Herbarium of the College of Science, Tōkyō Imperial University, and also in his own herbarium. He has been so fortunate as to find a few more examples of double stipules which are worth recording.

Galium kamtschaticum, Stell., is a strictly 'four-leaved' species. In a specimen of its typical form (*a. hirsutum*, Takeda),² collected by K. Endō in the Island of Shimushu, the most northerly of the Kuriles, the writer has found, at the uppermost node on the stem, one of the leaf-like stipules provided with two midribs, while the lamina is distinctly two-lobed. One of the lobes has only one of the lateral veins, while the other possesses both developed, as is usually the case in a stipule (Fig. 28).

In a specimen of a variety of the same species (*G. kamtschaticum*, Stell., *β oreganum*, Piper),³ collected by the writer on Mount Shirane, Nikkō, in July, 1905, a double stipule of a similar appearance has been observed (Fig. 29). In the two cases just mentioned, one of the two lobes of the stipule is larger than the other, and possesses both of the lateral veins belonging to this particular lobe developed. In the smaller lobe, on the other hand, the lateral vein that is situated on the outer margin of the double stipule is properly developed, while the other is not produced. In another specimen of the same series there is a double stipule in which both of the lobes are practically equal in size. In this case there is no lateral vein in the middle region of the stipule. These two cases of double stipules have also been found at the uppermost node on the stem.

¹ This communication is an addendum to the author's paper which appeared under the same title in *Ann. of Bot.*, vol. xxx, p. 197.

² In *Tōkyō Bot. Mag.* xxiv, p. 65 (1910).

³ Cf. *ibid.*, p. 66.

[*Annals of Botany*, Vol. XXX, No. CXX, October, 1916.]

The second species in which double stipules have been found is *Rubia grandis*, Kom.¹ The specimen was collected, also by the writer, along the road between Otchishi and Komp-moi, Yezo, in August, 1909. As a rule this species has four or five 'leaves' to the whorl. In this particular specimen all the foliar organs had decayed away from the first two nodes at the base of the stem, which is continuous with a rhizome. The third node has four 'leaves', the fourth five, and the fifth again possesses four. At the sixth node there are four 'leaves', but one of the stipules is evidently of a double

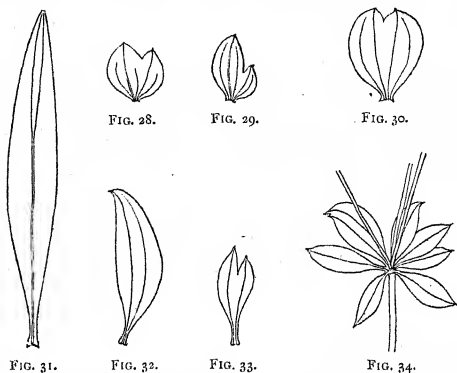


FIG. 28. *Galium kamtschaticum*, Stell., α *hirsutum*, Takeda. Double stipule, nat. size.
 FIGS. 29, 30. *Galium kamtschaticum*, Stell., β *oregonum*, Piper. Double stipules, nat. size.
 FIGS. 31, 32. *Rubia grandis*, Kom. Double stipules, nat. size.
 FIG. 33. *Asperula odorata*, L. Double stipule, nat. size.
 FIG. 34. *Asperula odorata*, L. Showing a false whorl with a double stipule, nat. size.

nature, being provided with a forked midrib, while its apex is hardly bidentate (Fig. 31). The seventh node is again four-membered, and both of the stipules are normal and smaller than the true leaves in the same whorl. At the eighth node there are on one side of the stem two small stipules, and on the other a double stipule which is nearly three times as large in area as one of the former (Fig. 32). At the next node there stands again a double stipule, but since all the other foliar organs but one true leaf of this whorl had been completely destroyed, apparently by insects, nothing further can be ascertained.

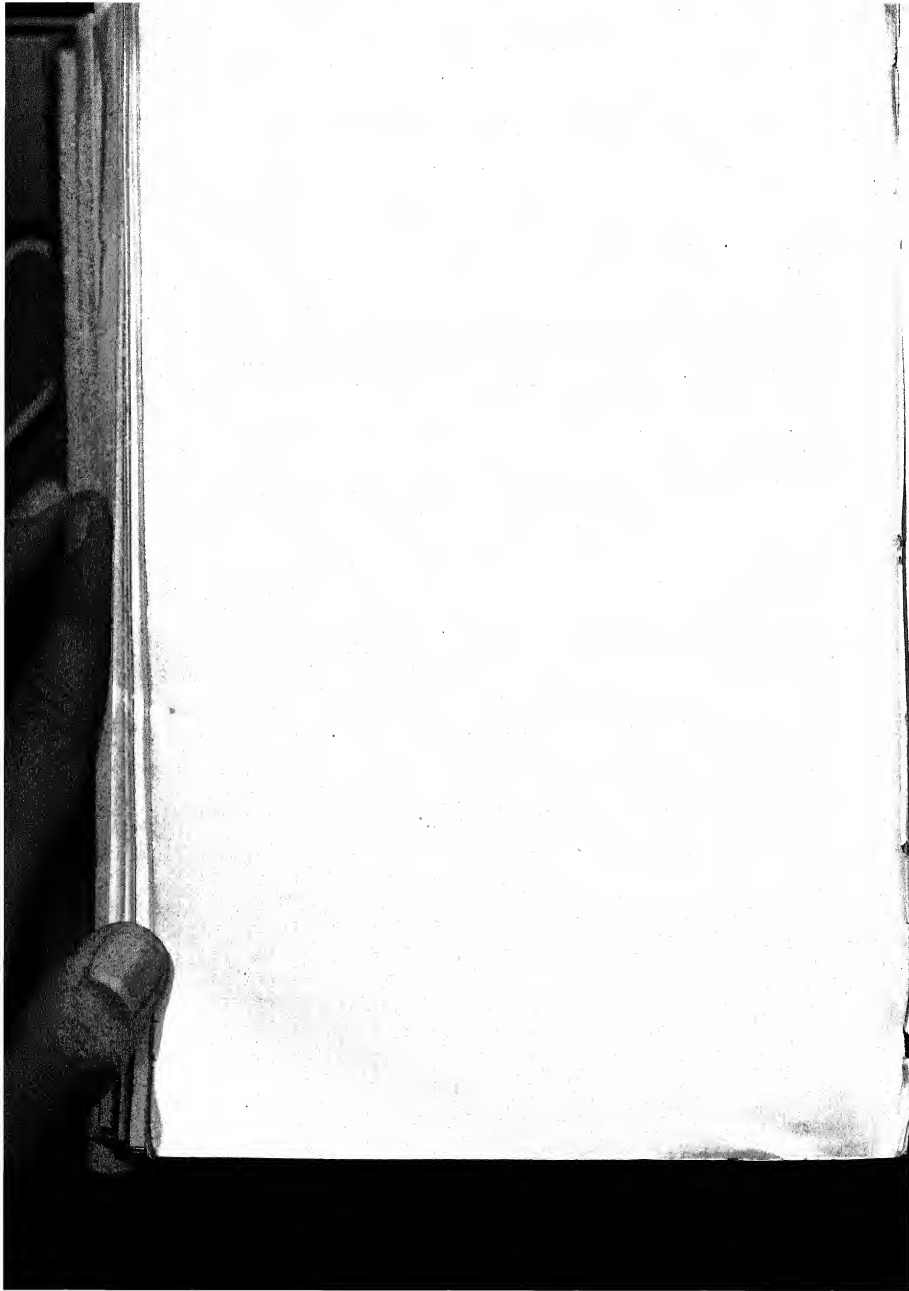
A series of specimens of *Asperula odorata*, L., which had been gathered by the writer himself near Satporo, Yezo, in June, 1906, affords

¹ Flora Manshuriae, p. 487 (1907). The plant was first described as a variety (var. *grandis*) of *Rubia tatarica* by Fr. Schmidt, Reisen im Amurl. n. d. Insel Sachalin (1868).

further instances of double stipules. One of the specimens has at the second node from the base of the stem two true leaves and two leaf-like stipules, one of which is provided with two midribs, the apex being at the same time fairly deeply two-lobed (Fig. 33). The whorl at the third node is six-membered, that at the fourth node eight-membered, and the ninth (the last) node has seven 'leaves'. Another specimen presents a feature of considerable interest. At the uppermost node on the stem, from which three peduncles spring, there are seven 'leaves', two being true leaves, each of which bears a branch in the axil, and the rest being leaf-like stipules. On one side of the stem, between the opposite true leaves, there are present three stipules. On the other side there are only two, but one of these is of a double nature, being furnished with two midribs and a shallowly indented apex (Fig. 34).

The points of interest are in the first place that the double stipule corresponds with two of the three stipules on the opposite side of the stem, and clearly indicates, as in the case of *Didymaea mexicana* (vide Ann. of Bot., vol. xxx, pp. 203-4), the probable mode by which the stipules at a node increase in number. In the second place, it affords the only instance, so far as the writer has observed, of the occurrence of a leaf-like double stipule in a whorl of more than five members.

It has also been found that in *Galium paradoxum* five-membered whorls with three leaf-like stipules are seldom produced.



NOTE.

NOTE ON A SPORELING OF PHYLLOGLOSSUM ATTACHED TO A PROTHALLUS.—All attempts to raise prothalli of *Phylloglossum* from spores having so far failed, we are dependent upon material collected in the field. In 1901 a preliminary account of some New Zealand specimens was given by Professor Thomas, but his paper contained no figures.¹ Since that date no further observations have been published, and the occurrence of a prothallus among some recent Australian material seems worth recording. The prothallus in question was attached to a young sporeling, which possessed a single leaf 8 mm. long, a slender root, and a small storage tuber (Fig. A).² The plant was embedded in a mass of peat, which contained other small plants, but these were all developed from storage tubers formed in the previous season.

The prothallus is a small tuberous body, about 2 mm. in diameter, the upper end being slightly extended to form a crown. In form and size it recalled the 'shorter thick-set prothalli' described by Professor Thomas. The preservation of the tissues was poor, but it was clear that the prothallus was of a relatively unspecialized type. It consisted mainly of large thin-walled cells, some of which had grown out to form rhizoids.

A notable feature of the prothallus was its association with fungal hyphae. Among the outer layers of cells, and occasionally among the rhizoids, relatively coarse, non-septate hyphae were found. These hyphae were probably the mycelium of some saprophytic fungus, which had helped to bring about the decay of the prothallus. Slender hyphae, septate and frequently coiled, occurred in abundance in the central region of the prothallus, and in sections through the crown (Fig. B, *fr*). Occasionally they showed a tendency to fragment, and in some sections they were associated with groups of deeply staining bodies. Thick-coated resting spores, formed either singly or in pairs at the ends of slender hyphae, occurred towards the edge of the prothallus. It was impossible to observe the relation between the fungal hyphae and the individual cells of the prothallus, but there seems little doubt that the thin septate type belonged to an endophytic fungus such as Professor Thomas described.

Professor Thomas found the sexual organs of *Phylloglossum* on the crown of the prothallus. In the specimen here described, the upper part of the prothallus was decayed and crushed, and its structure was difficult to determine, but in two consecutive sections a rounded mass of small cells was observed on the edge of the crown (Fig. B, A). The specimen had been embedded, cut transversely,

¹ Thomas, A. P. W.: Preliminary Account of the Prothallus of *Phylloglossum*. Proc. Roy. Soc., vol. lxi, 1901-2, p. 285.

² Thomas states that he has seen no formation of root during the first year of growth. Loc. cit., p. 288.

and stained with aniline safranin and light green. The safranin had collected in the corners of these cells, a fact which helped to distinguish them from those of the surrounding tissue. Though nothing can be definitely stated, it is possible that they were the cells of an immature antheridium.

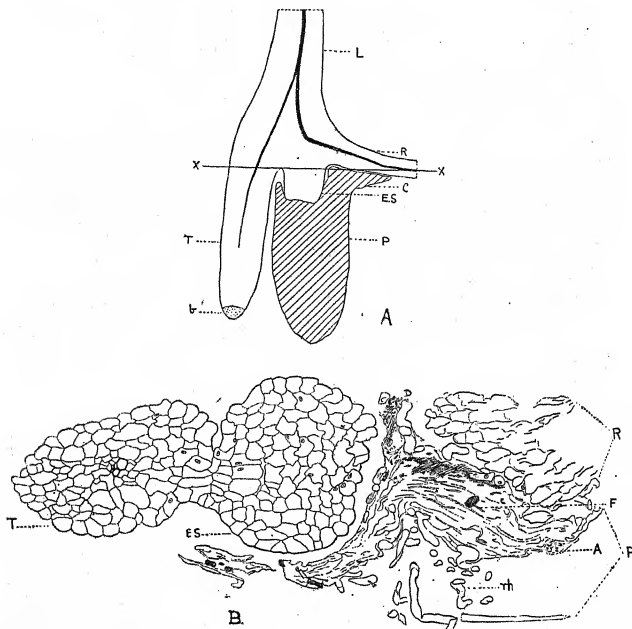


FIG. A. Diagram constructed from serial transverse sections, to show the sporophyte and the prothallus in vertical longitudinal section. $\times 23$.

FIG. B. Transverse section through the sporophyte and part of the prothallus, drawn from two consecutive sections at the level marked $\times \times$ in diagram A. $\times 53$.

L=leaf; R=root; T=storage tuber; b=bud; ES=embryonic swelling; P=prothallus; C=crown of prothallus; F=fungal hyphae; A=antheridium; rh=rhizoids; D=debris. The sections which were lost occurred just above the level marked $\times \times$.

Besides the leaf, root, and storage tuber already referred to, the sporophyte possessed a tuberous mass of tissue, which was partly immersed in the prothallus and partly free. This body consisted of parenchymatous cells, which differed from those of the gametophyte in that they contained no fungal hyphae, and were in a better state

of preservation (Figs. A and B, e.s.). The morphology of this part of the sporeling presents some difficulty. Having only a single specimen we are without data of development, and not even could the position of the archegonium neck be discovered, since one or two sections of the microtomed series were unfortunately lost. Two alternative views are possible. The tuberos body may be either a local swelling in the hypobasal region of the embryo, comparable with the foot found in an embryo of the *L. clavatum* type, or it may have arisen in the epibasal part of the embryo, and therefore correspond with the protocorm of *L. cernuum*. In the specimen examined there is nothing against regarding the swollen part of the sporeling as a foot, except the fact that it is partly extraprothallial, and even this might be due to decay and shrinkage in the gametophyte. On the other hand, the account given by Professor Thomas seems to support the second alternative. This observer states that the early stages of embryogeny in *Phylloglossum* are much like those in *L. cernuum*, and he describes a condition in which the embryo, 'a short cylindrical body, bluntly pointed at both ends', is attached to the prothallus by a foot which is lateral in position. It is possible, therefore, that in the specimen described above, a foot, relatively small and lateral to the sporeling, was present in the sections which were lost. If this be so the bulky mass of tissue half buried in the prothallus must correspond, not with the foot of *L. clavatum*, but with the protocorm of *L. cernuum*. It differs from this organ, however, in possessing no rhizoids and no endophytic fungus, and in being only partially free from the prothallus.¹

Although the morphology of the embryonic swelling must be left an open question until further observations can be made, the specimen seemed worthy of record because the sporeling is found to possess both an embryonic swelling and a characteristic storage tuber. Thus it demonstrates (a) that *Phylloglossum* falls into line with one or other of the Lycopodian series with an embryonic swelling, and (b) that the annual storage tuber is of entirely independent origin.

¹ For discussion of the problem see Bower, *Origin of a Land Flora*, chap. xxvi, where also further references to the literature on the subject are given. I regret that all reference to Professor Bower's treatise was omitted by mistake in my paper on the Morphology of *Phylloglossum*, *Ann. of Bot.*, vol. xxx, p. 315.

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